Reaction Mechanism between Estradiol Benzoate and a Strongly Basic Anion Exchange Resin in Methanol and Ethanol

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In a previous paper[1] it was shown that estradiol benzoate is completely saponified by a strongly basic anion exchange resin and that estradiol is taken up quantitatively by the resin. Further experiments carried out in this investigation have shown that, in contrast to estradiol, the benzoic acid portion is not retained when estradiol benzoate is passed through the resin bed in absolute alcohol solution (methanol or ethanol), but instead is converted completely into alkyl benzoate which passes into the effluent.

EXPERIMENTAL

Materials and analysis. Estradiol 3-benzoate was obtained from Usines Chimiques des Laboratoires Français (UCLAF). Methyl benzoate and ethyl benzoate (Merck, Darmstadt), redistilled *in vacuo*, b.p. 73.5–74.0°/8 mm. and 79.5–80.5°/5 mm. respectively, were used as reference standards. Methanol (Merck, anal. grade) and ethanol (A/S Danisco, Copenhagen), having a water content of 0.04% and 0.44% (by weight) respectively, according to the Karl Fischer method, were used as solvents.

The experiments were performed with the commercial resin Dowex 1. The resin was ground to a particle size of 0.15–0.30 mm., screened, purified, and converted into the hydroxyl ion form in the same manner as in the foregoing investigation.[1] The ion exchange columns were of a standard type, measuring 6 × 110 mm. The columns were placed in a water thermostat adjusted to 35 ± 0.1°C. After the columns were washed with about 50 ml. of the solvent *i.e.* until no absorption could be observed in the ultraviolet region, estradiol benzoate solutions were passed through and the columns were afterwards washed out to obtain an effluent volume of 50 ml. The flow rate in all experiments was about 1 ml./min.

Methyl benzoate and ethyl benzoate were determined in the ultraviolet region, their absorption maxima being 226 mμ. For the detection of estradiol the reaction for phenols by Folin and Ciocalteau was used.[1] A Unicam SP 500 Spectrophotometer, fitted with matched 1-cm. absorption cells, was used for the measurements.

RESULTS AND CONCLUSIONS

Table I shows the conversion of various amounts of estradiol benzoate into methyl and ethyl benzoate. The conversion is complete within the limits of the experimental errors. In all cases not more than 0.1% of estradiol could be detected in the effluent, *i.e.* the uptake of estradiol was quantitative.

TABLE I

CONVERSION OF ESTRADIOL BENZOATE IN ABSOLUTE METHANOL AND ETHANOL

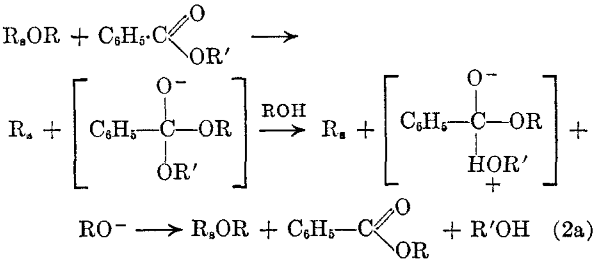
Added	*Found in effluent*			
Estradiol benzoate	Methyl benzoate in methanol		Ethyl benzoate in ethanol	
10^{-1} Mole	10^{-6} Mole	Conversion, %	10^{-6} Mole	Conversion, %
7.98	7.92	99.2	7.94	99.5
13.31	13.18	99.0	13.31	100.0
26.62	*26.85*	*101.0*	*27.02*	*101.5*
53.24	52.49	98.6	53.77	101.0

Should the resin phase contain hydroxyl ions, the conversion of estradiol benzoate would not be possible. Under such conditions estradiol benzoate would be saponified, yielding benzoic acid which should be retained by the resin. Therefore, and in agreement with other experimental data, the following explanation seems obvious.

When alcohol comes into contact with the resin the reaction (1) takes place and the hydroxyl ions undergo a displacement by alkoxide ions. This reaction has been verified by several investigators in alcohol solution containing alkali hydroxide.[2–4]

$$ROH + OH^- \underset{(\leftarrow)}{\longrightarrow} RO^- + H_2O \qquad (1)$$

After washing the resin with a given amount of alcohol all the hydroxyl ions are displaced. Finally, when estradiol benzoate solution is passed through, the reaction proceeds in the following way:



$$R_sOR + C_6H_5\cdot C\begin{matrix}\nearrow O\\ \searrow OR'\end{matrix} \longrightarrow R_s + \left[C_6H_5-\overset{\displaystyle O^-}{\underset{\displaystyle OR'}{\overset{|}{\underset{|}{C}}}}-OR\right] \xrightarrow{ROH} R_s + \left[C_6H_5-\overset{\displaystyle O^-}{\underset{\displaystyle \underset{+}{HOR'}}{\overset{|}{\underset{|}{C}}}}-OR\right] + RO^- \longrightarrow R_sOR + C_6H_5-C\begin{matrix}\nearrow O\\ \searrow OR\end{matrix} + R'OH \qquad (2a)$$

$$R_sOR + R'OH \longrightarrow R_sOR' + ROH \qquad (2b)$$

where R_s^+ is the resin cation, R the alkyl radical (methyl or ethyl), and R'OH is estradiol.

In the first step (2a) estradiol benzoate is converted into alkyl (methyl or ethyl) benzoate and estradiol is formed. The mechanism of this reaction can be considered as alcoholysis.[5] In the second step (2b) estradiol is take up by the resin and alcohol is formed.

In consequence of what has been stated above, an increase in the water content would move the state of the equilibrium in reaction (1) to the hydroxyl ion side. Thus, all the hydroxyl ions would not be displaced if alcohol containing water is passed through the resin, and the saponification of estradiol benzoate would partly yield benzoic acid. That this is actually the case is shown in Table II.

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[1] E. Sjöström and L. Nykänen, *Suomen Kemistilehti*, **29B**, 23 (1956).

[2] D. Williams and R. W. Bost, *J. Chem. Phys.*, **4**, 251 (1936).

[3] E. F. Caldin and G. Long, *J. Chem. Soc.*, 3737 (1954).

[4] A. Koivisto, *Acta Chem. Scand.*, **8**, 1218 (1954).

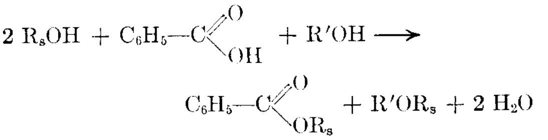
[5] A. E. Remick, *Electronic Interpretations of Organic Chemistry*, 2nd ed., John Wiley & Sons, New York, 1949, pp. 408-419.

TABLE II

Conversion of Estradiol Benzoate (13.31×10^{-6} Mole) in Methanol-Water and Ethanol-Water Mixtures

Alcohol concentration per cent by weight		*Found in effluent*			
		Methyl benzoate in methanol		Ethyl benzoate in ethanol	
Methanol	Ethanol	10^{-6} Mole	Conversion, %	10^{-6} Mole	Conversion, %
99.96	99.56	13.18	99.0	13.31	100.0
90	90	12.44	93.5	11.26	84.6
80	80	10.63	77.9	9.68	72.7
70	70	8.20	61.6	8.32	62.5
—	60	—	—	7.21	54.9
—	50	—	—	6.50	48.8

It can be noted that the greater the amount of water, the less is the conversion into methyl or ethyl benzoate, *i.e.* the more benzoic acid is formed and retained by the resin. The first step of the reaction between estradiol benzoate and the resin in the hydroxyl form can be represented by the equation (2a), substituting H for R. In this step estradiol benzoate is saponified to estradiol and to benzoic acid (instead of alkyl benzoate). The mechanism of this reaction is the same as in a normal ester hydrolysis.[5] In the second step benzoic acid and estradiol are taken up by the resin:



$$2\,R_sOH + C_6H_5{-}C(=O)OH + R'OH \longrightarrow C_6H_5{-}C(=O)OR_s + R'OR_s + 2\,H_2O \quad (2c)$$

Thus, whether the reaction of an ester (estradiol benzoate) with an anion exchange resin in alcohol-water mixture lies in favor of alcoholysis or hydrolysis, seems to depend primarily upon the equilibrium of the reaction (1). Studies of this equilibrium and its influence upon the ester hydrolysis in anion exchangers are now in progress and will be published in forthcoming papers.

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Some Hydroxyfluorene Derivatives[1]

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3-Hydroxyfluorene has been prepared by two different procedures in the literature. Harradence and Lions[3] have prepared it from 1-indanone in 5 steps. The procedures of Ullmann and Bleier[4] and Lothrop[5] give the compound in 6 steps starting from anthranilic acid. Based on the work of Errera and La Spada[6] a three-step procedure starting from 1,3-indandione has been developed in our laboratory. Using the same procedure 2-acetyl-3-hydroxy-9-fluorenone and 2-ethyl-3-hydroxyfluorene have been prepared. Various other types of 3-hydroxyfluorene derivatives could be made available by this method.

The correct assignment of structure for 3-hydroxyfluorene and 2-ethyl-3-hydroxyfluorene is evident from the close u.v. spectral resemblance of these compounds to 3-methoxyfluorene,[7] Table I. 2-Ethyl-3-hydroxyfluorene and 2- and 3-hydroxyfluorene have an O–H stretching frequency at 2.80 μ in carbon tetrachloride solution. The ethyl derivative has a greatly strengthened aliphatic C—H stretching frequency at 3.4–3.5 μ.

2-Acetylamino-3-hydroxyfluorene has been synthesized in 30% over-all yield from 3-hydroxyfluorene by a procedure that involved chromatography.[8] As it was necessary to prepare a large quantity of the acetylaminohydroxy derivative, a simplified procedure was developed involving coupling of benzenediazonium chloride with 3-hydroxyfluorene, and reduction to the amine hydrochloride followed by acetylation. Starting from 3-hydroxyfluorene the over-all yield was about 68%.

The structure of the 2-acetylamino-3-hydroxyfluorene was based on spectroscopic data and melting point. The ultraviolet-visible absorption spectrum of the phenylazo-3-hydroxyfluorene is entirely different from the spectrum of 2-ethyl-3-hydroxy-4-phenylazofluorene in alcohol, Fig. 1. This and the following data clearly show that the phenylazo group is in the 2-position. The 2-acetylamino-3-hydroxyfluorene obtained from the 2-phenylazo derivative had ultraviolet absorption maxima and minima and molar extinction coefficient values almost identical with that reported by the Weisburgers.[8]

Nitration of 2-acetoxyfluorene gave the 7-nitro derivative. This procedure gave a much purer product than was obtained from 7-nitro-2-aminofluorene,[9] for the 7-nitro-2-hydroxyfluorene derived from the latter compound was found to contain a persistent impurity.

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(9) Weisburger and Weisburger, *J. Chem. Soc.*, 758 (1954).