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Some Nitrated Derivatives of Estriol

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The synthesis, the infrared and ultraviolet absorption spectra as well as the thin layer chromatographic properties of 2-nitroestriol and 2,4-dinitroestriol are described. The estrogenic potency of dinitrated estrone and estriol is reported.

In connection with investigation of the transformation on the aromatic ring of steroids a series of nitrated derivatives of estrone and estradiol have been published and the estrogenic activity of some of the substances has been reported.

In 1949 Niederl and Vogel[1] reported the synthesis of 2-nitroestrone and in 1955 Hillmann-Elies *et al.*[2] published the synthesis of 2- and 4-nitroestrone, respectively. Subsequent investigations[3,4] demonstrated, however, that these compounds have the reverse structure, *i.e.* the formerly supposed 2-nitroestrone is in fact 4-nitroestrone and *vice versa*. In 1956 Werbin and Holoway[3] synthetized and described the correct structure of the two above-mentioned compounds and at the same time prepared 2,4-dinitroestrone and 2,4-dinitro-17-deoxoestrone. In the same year Werbin[5] also reported the synthesis of 2,4-dinitroestradiol-17β. Patton[6] in 1959 described the synthesis of 2-nitro- and 4-nitroestradiol-17β over two routes: by the selective reduction of the corresponding nitroestrone and by the direct nitration of estradiol-17β. It is to be noted that this author has preferred the first method (the nitration of estrone followed by sodium borohydrid reduction of the mono-nitro products), because the 2- and 4-nitroestrone are more easily separated than a mixture of 2- and 4-nitroestradiol. The synthesis of the two missing mono-nitro-17-deoxoestrones has been described by Werbin *et al.*[7] in 1962, although the infrared absorption spectra of these two compounds had already been reported.[4] Patton and Dmochowski[8] have described the estrogenic activity of dinitrated estradiol and found this to be very low in comparison with that of estradiol.

It was of interest to us to investigate the nitration of estriol under similar conditions (direct nitration) and to assess the influence of nitration upon the estrogenic activity.

When using one equivalent of concentrated nitric acid a mixture of 2-nitroestriol (I) and 4-nitroestriol (II) resulted, together with a small amount

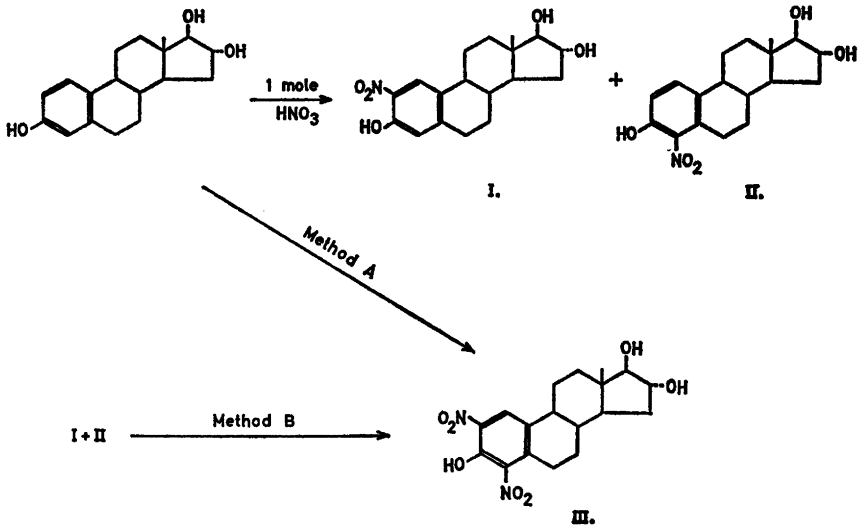

Fig. 1. The nitration-procedure of estriol.

of 2,4-dinitroestriol (III) and traces of two not identified impurities (Figs. 1 and 2). By preparative chromatography a pure, homogeneous compound was isolated from the mixture, which in all probability consists of 2-nitroestriol (Fig. 2). With two moles of concentrated nitric acid only 2,4-dinitroestriol (III) was isolated. Further nitration of the mixture of I and II resulted in the formation of the same product (III).

Ultraviolet and infrared absorption spectra were recorded for the obtained nitro-compounds and compared with ultraviolet data given by Werbin and

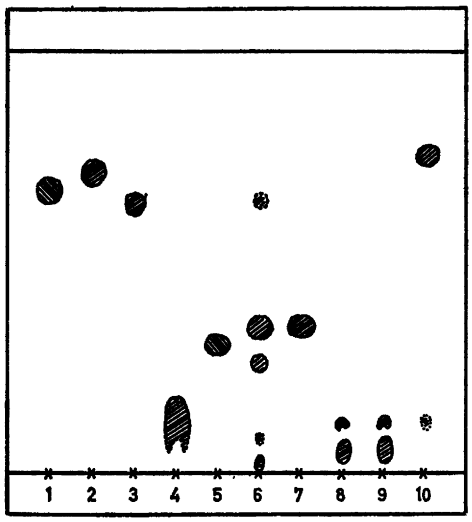

Fig. 2. Thin layer chromatography of some nitroderivatives of estrone and estriol on activated silica gel in acetone + chloroform (1:1). The nitroderivatives gave strong, yellow to orange spots after treatment with NH_3-vapor. Estrone and estriol were made visible by spraying with H_2SO_4 in ethanol and subsequent heating to 105°C.

1. Estrone
2. 2-Nitroestrone
3. 4-Nitroestrone
4. 2,4-Dinitroestrone
5. Estriol
6. Mixture obtained by nitration of estriol with one mole HNO_3
7. 2-Nitroestriol
8. 2,4-Dinitroestriol (method A)
9. 2,4-Dinitroestriol (method B)
10. 2,4-Dinitroestriol 3,16α, 17β-triacetate

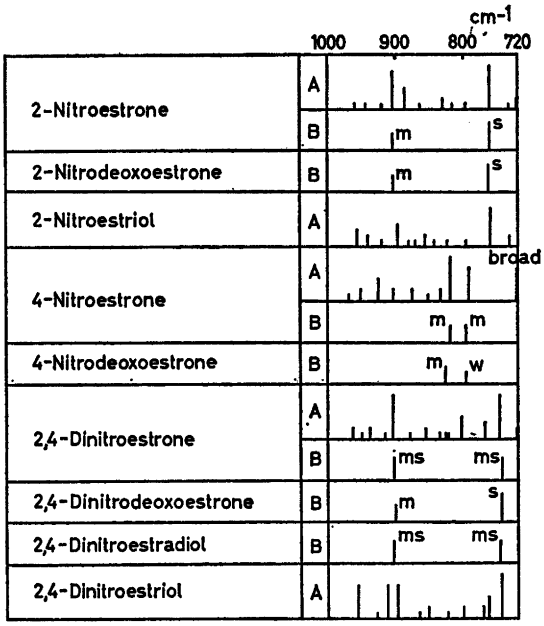

Fig. 3. Infrared absorption bands in the 720-1000 cm^{-1} region. Spectra were taken up with a Perkin-Elmer model 21 spectrophotometer with the potassium bromide technique. A=The present investigation. B=Characteristic bands given by Pickering and Werbin;[4] s, m, and w=strong, medium, and weak intensities.

Table 1. Ultraviolet absorption maxima and molar extinction coefficients of some nitro-derivatives of estrogens.[a]

Compound		maxima mμ	ε	maxima mμ	ε	maxima mμ	ε
2-Nitroestrone[b]	A	293	8120	363	3760		
—,,—	B	293-294	8220	364-366	3710		
4-Nitroestrone[b]	A	277	1777				
—,,—	B	278	1720				
2-Nitroestriol	A	290	7310	363	3350		
2,4-Dinitroestrone[b]	A	277	6870	351	3470		
—,,—	B	276	6890	353	3405	430	1050
2,4-Dinitrodeoxoestrone	B	278-279	6760	353-354	3575		
2,4-Dinitroestriol[c]	A	275	6600	346	3280	420	1187

A = The present investigation.
B = Values given by Werbin and Holoway.[3]

[a] Procedure: Spectra were taken up with a Beckman DB recording spectrophotometer with ethanol as solvent.
[b] Prepared according to Werbin and Holoway.[3]
[c] Prepared according to method A.

Holoway[3] and with infrared data given by Pickering and Werbin[4] (Table 1 and Fig. 3). It was shown that the mononitroestriol isolated by preparative chromatography must be the 2-nitroestriol. The data of dinitroestriol were in good agreement with those from other dinitroderivatives.

We have tested * two dinitrated estrogens, namely 2,4-dinitroestrone and 2,4-dinitroestriol for estrogenic activity according to the bioassay method of Allen-Doisy** using vaginal smears in adult spayed mice and have found that these compounds exhibit an extremely low estrogenic activity, a finding that is associated with a high toxicity (Table 2).

Table 2. Estrogenic effect of dinitrated estrogens.[a]

Compound	No. of mice[b]	Dose in μg[c]	Response in per cent
Estrone	8	0.05	50
—,,—	12	0.10	100
Estriol	9	3.0	11
—,,—	9	6.0	100
2,4-Dinitroestrone	14	10.0[d]	7
2,4-Dinitroestriol	13	80.0[e]	15

[a] Procedure: The mice were injected 3 days with respective doses in a total volume of 0.8 ml. Vaginal smears were taken on the 4th, 5th, 6th, and the 7th day after the first injection.
[b] Adult, castrated, female mice.
[c] The substances were injected in propylene glycol solution.
[d] All animals died when given 100 μg.
[e] All animals died when given 800 μg.

EXPERIMENTAL

All melting points were determined on a Kofler block and are uncorrected. Rotations were determined in ethanol + chloroform (1:1) in a 10 cm tube.

Mononitroestriols. Estriol (1.44 g, 0.005 mole) was dissolved in 90 ml 95°C acetic acid and allowed to cool to 60°C. 0.375 ml of nitric acid (d 1.40) was added with stirring to the clear solution in 4 portions during 1 h. After keeping for 30 h at room temperature, the solution was filtered and added with stirring dropwise into 1750 ml ice-water. After keeping overnight at 5°C the amorphous precipitate was filtered and washed with cold water and dried over P_2O_5 *in vacuo*. Yield: 1.20 g. The yellow substance had no real melting point and consisted substantially of a mixture of mononitroestriols (Fig. 2). It was chromatographed on Merck aluminium oxide (standardized for chrom. adsorption analysis according to Brockmann). The column was eluted with benzene + methanol (98:2, 95:5 and 90:10, successively). By working-up of the different fractions we succeeded in obtaining a crystalline substance from the middle fraction in a yield of 220 mg, which after recrystallization from aqueous ethanol gave 2-nitroestriol (I), as a yellow product (Figs. 2 and 3). M.p. 172—175.5°C, $[\alpha]_D^{25}$ +100° (c=0.96). (Found: N 3.70. Calc. for $C_{18}H_{23}O_5N$: N 4.20).

2,4-Dinitroestriol (III). (A) Direct nitration of estriol. Estriol (1.44 g, 0.005 mole) was dissolved in 70 ml of hot acetic acid and allowed to cool to 75°C. Then 0.75 ml of conc. nitric acid was added to the clear solution in 8 portions during 80 min with

* We are indebted to Dr. E. Diczfalusy, Hormon Laboratory, Department of Women's Diseases, Karolinska Sjukhuset, Stockholm, for carrying out the bioassays.
** Emmens, C. W., ed. *Hormone Assay*. Academic Press, N.Y. 1950.

stirring. After the last portion the reaction mixture was continuously heated 1 h at 75°C and thereafter kept for 20 h at room temperature. The yellowish red solution was filtered and added dropwise to 1200 ml of ice-water while stirring. An amorphous precipitate formed, which after keeping for several hours at 5°C was filtered and washed with 6 % acetic acid and cold water and dried over P_2O_5 *in vacuo*, yield: 700 mg, m.p. 200–210°C (dec.). After twice recrystallization from aqueous ethanol (with charcoal treatment) a yellow powder was obtained. It had a m.p. 226–230°C (dec.) (over 200°C strong darkening), $[\alpha]_D^{25}+123°$ ($c=1.07$). By thin layer chromatography it was found to contain a small amount of an impurity with a little higher R_F-value (Fig. 2). (Found: C 57.0; H 5.90; N 7.20. Calc. for $C_{18}H_{22}O_7N_2$: C 57.13; H 5.86; N 7.44). 2,4-Dinitroestriol was not quite stable on storage at room temperature.

(B) Conversion of the mixture of 2-nitro- and 4-nitroestriol to 2,4-dinitroestriol. The mixture of I and II (660 mg) was dissolved in 40 ml of hot acetic acid and nitrated with 0.375 ml of concentrated nitric acid under the same conditions as by the direct nitration of estriol. After similar processing a precipitate was obtained, which was difficult to filter and therefore extracted 3 times with ether and washed twice with cold water. After drying over anhydrous sodium sulphate the solvent was removed *in vacuo*. The resulting oily residue was crystallized with charcoal treatment from aqueous ethanol. This resulted in a yellow powder melting at 220°C (decomp.); yield: 520 mg. Recrystallization from aqueous acetic acid afforded a sample, m.p. 228–232°C (decomp.) (over 200°C strong darkening). This substance was identical with the substance III, obtained by direct nitration of estriol (Fig. 2); mixed m.p. 226–230°C (decomp.).

2,4-Dinitroestriol 3,16α,17β-triacetate. A mixture of III (1.20 g, 0.0036 mole) and *p*-toluenesulfonic acid (1.0 g) was heated in 40 ml acetic anhydride on a steam bath until a clear solution was obtained. After 20 h at room temperature the solution was cooled and to the somewhat opalescent cold solution a mixture of 420 ml water and 30 ml pyridine was added. After keeping for several hours a fine precipitate formed, which was filtered and washed with 6 % acetic acid and with water and dried over P_2O_5 *in vacuo*. Yield: 1.2 g. After recrystallization from methanol with charcoal treatment a pure sample was obtained with m.p. 211–212.5°C, $[\alpha]_D^{25}+61°$ (c=0.97). By thin layer chromatography it was found to contain traces of the same impurity as the 2,4-dinitroestriol (Fig. 2). (Found: Acetyl 25.55; N 5.69. Calc. for $C_{24}H_{28}O_{10}N_2$: Acetyl 25.59; N 5.55).

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