

The Preparation and Pharmacology of Some 3-Desoxyestratrienes. Lipid-Shifting and Estrogenic Effects

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Estratrien-17-one and its 3-methyl and 4-methyl derivatives have been converted into a number of new 17-substituted estratrienes lacking the usual phenolic oxygen function in the A-ring. 2-Methylestratrien-17-one and 3-methylestratrien-17-one, previously unknown, were prepared by known methods from estrone and 19-nortestosterone, respectively. The 3-desoxy- and A-ring-methylated 3-desoxyestratrienes are far less potent estrogens than the 3-phenols, being closer in this respect to the 3-methyl ethers, based on a mouse uterine growth assay. In lowering of the plasma cholesterol-phospholipid ratio in cholesterol-fed cockerels, a known effect of the natural estrogens, removal of the phenolic hydroxyl has little or no effect. Replacement with an A-ring methyl group at position 2, 3, or 4 lowers this lipid-shifting potency, though this effect is not as great as the decrease in estrogenicity.

In view of the known low incidence of coronary atherosclerosis in premenopausal women and a possible direct relationship to estrogenic hormone levels,¹ it seemed desirable to prepare some simple 3-desoxyestratrienes for pharmacological testing.

3-Desoxyestrone and 3-desoxyestradiol have been shown to have weak estrogenic activity and normal estrogenic dose-response curves at high dose levels.² It was hoped that suitable analogs would retain desirable antiatherogenic properties³ with loss of the undesirable feminizing effects of this class of compounds.

Reduction in the plasma cholesterol-phospholipid ratio is achieved with estrogens and is associated with inhibition of coronary lesions in cholesterol-fed cockerels.³ Also, in rats, a serum lipid elevation is observed with estradiol benzoate⁴ and this may be due to increased phospholipids. Efficacy of estrogens in clinical states related to atherosclerosis and associated with hypercholesterolemia has also been observed, though the treatment also resulted in undesirable estrogenic side effects.⁵

It was the intent of this study to find compounds which would lower the plasma cholesterol-phospholipid ratio, but lack altogether, or have only very weak, estrogenic activity.

A second point of interest concerned whether compounds for evaluation would be impeded estrogens,² that is, have shallow estrogenic dose-response curves. This point has not as yet been thoroughly studied,

though it is probable that, for reasonable hope of clinical utility, weak estrogens with desirable lipid-shifting properties should also be impeded estrogens. The latter property is shown by 16 α -methylestriol 3-methyl ether^{1,6a} and this is in agreement with previous studies on structural requirements² and with other studies in our laboratories.^{6a} Clinical trials with this compound and with 16 α -chloroestrone 3-methyl ether have shown diminished, though occasionally significant, feminizing effects at the dose levels which were used.^{1a,6a}

In passing, it should be noted that the designation of estrogenic side effects as occurring in the male is probably determined by the intensity of such effects rather than simply by their presence or absence. It is known that estrogens are produced by the human male and that they are present in the general circulation. Therefore, effects which are especially noted with large doses of estrogen are normally operative in some degree. Considering the sensitivity of recently developed estrogen assays,^{6b} it may be possible to make correlations between the incidence of vascular disease in males and average individual estrogen levels.

Pharmacological Results.—Cholesterol and phospholipid were determined^{7,8} in a short-term test using cholesterol-fed cockerels.¹ The estrogenic potency was obtained by a modification of the method of Rubin, *et al.*, utilizing uterine growth measurements in mice.^{9,10}

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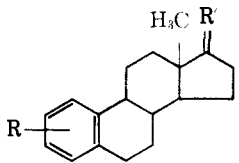
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(8) W. M. Sperry, *Ind. Eng. Chem. Anal. Ed.*, **14**, 88 (1942).

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TABLE I
 LIPID-SHIFTING^a AND ESTROGENIC^b ACTIVITIES AND THEIR RATIOS^c


R ^c	3-OH	3-OCH ₃	H	3-CH ₃	4-CH ₃	2-CH ₃	$\frac{O}{3-OP_1(OEt)_2}$
$\begin{matrix} R \\ \diagdown \\ \text{C} \\ \diagup \\ \text{=O} \end{matrix}$	100 ^a	285	130	30	15	<5	20
	100 ^b	29	10	1.6	0.1	<0.01	10
	1 ^c	10	13	19	150		2
$\begin{matrix} \text{OH} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{H} \end{matrix}$	175	205	120	25	10		15
	370	11	10	2	0.3		10
	0.5	20	12	13	30		1.5
$\begin{matrix} \text{OAc} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{H} \end{matrix}$	320	>100	200	10	10		
	170	3.2	30	1	0.1	<0.01	
	2	>30	7	10	100		
$\begin{matrix} \text{OH} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{C}\equiv\text{CH} \end{matrix}$	90	170	150	25	<10		
	770	50	>10	15	0.25		
	0.1	2	<15	2	<40		
$\begin{matrix} \text{OH} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{CH}=\text{CH}_2 \end{matrix}$		25	10	<10			
	100	6.5	0.1				
		4	100				
$\begin{matrix} \text{OH} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{C}_6\text{H}_5 \end{matrix}$	<5	<5	<5	<10	<10		
	1	0.04-1	0.1				
	<5	<125	<50				
$\begin{matrix} \text{OH} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{CH}_3 \end{matrix}$		<10	20		<10		
			>3		0.1		
			<7		<100		
$\begin{matrix} \text{OH} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{CH}_2-\text{CH}=\text{CH}_2 \end{matrix}$	20				<10		
	0.7						
	30						

^a The top figure for each compound is the relative lowering of the plasma cholesterol-phospholipid ratio (lipodiatic activity) in cholesterol-fed cockerels; estrone = 100; see ref. 1, 6a, 7, and 8. ^b The middle figure for each compound represents the relative estrogenic potency by mouse uterine assay; estrone = 100; see ref. 1, 6a, 9, and 10. ^c The bottom figure is the ratio of lipid shifting (lipodiatic) to estrogenic activity (a:b). A high ratio is theoretically desirable, representing a lowering of cholesterol-phospholipid which is greater relative to estrogenicity than the lowering observed with estrone; estrone = 1.

Table I gives the results obtained for series of 3-desoxy-, 3-desoxy-3-methyl-, 3-desoxy-4-methyl-, and 3-desoxy-2-methylestratrienes with varying substitution at C-17. Included for comparison are some 3-oxygenated estrogens, related 17-substituted derivatives, and their 3-methyl ethers. Some of the data for the 3-oxygenated compounds have previously been reported.^{1,6a}

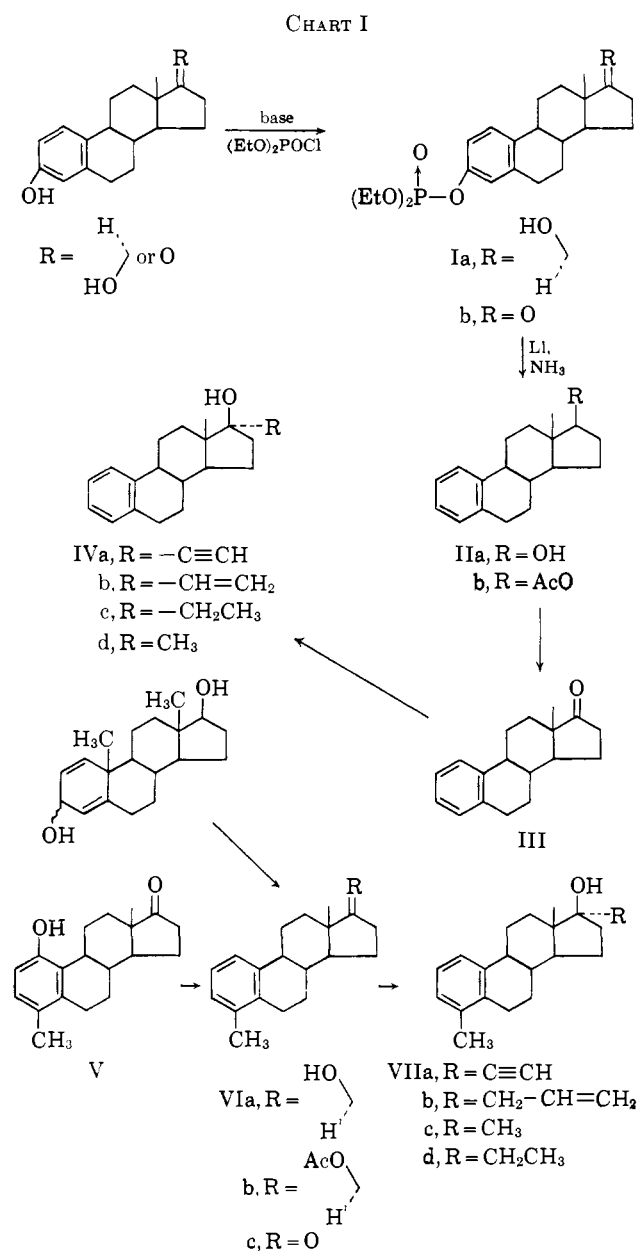
The lipodiatic¹¹-estrogenic ratio (a:b; see footnotes for Table I) for the standard (estrone) is arbitrarily assigned a value of 1. This ratio increases by a factor of 10 for the 3-methyl ether of estrone and by factors of 13 and 19, respectively, for the 3-desoxy and 3-methyl compounds. The 3-methyl compounds, however, show a significant lowering of both the lipodiatic and estrogenic activities. Thus, no desired separation of effects

is associated with these structural changes at position 3.

With 4-methylestra-1,3,5(10)-trien-17-one, lowering of the plasma cholesterol-phospholipid ratio is less than that observed with estrone by a factor of 6-7. The effect on estrogenic activity, however, is a reduction in response by a factor of 1000 compared to estrone. Thus, of the 17-keto compounds in Table I, the highest lipodiatic-estrogenic ratio occurs with 4-methyl substitution. As seen in Table I, this ratio appears to be generally greater for 4-methyl compounds than for the other desoxy derivatives.

3-Desoxy compounds of Table I which have lipodiatic-estrogenic ratios that may compare favorably with the 4-methyl series are the 3-desoxy-17-vinyl compound and the 2-methylestratrienes. The latter have potencies too low to make practical a complete evaluation of their lipodiatic-estrogenic ratios. It is possible, however, to predict to some degree what might be expected for these values based on removal of the phenolic hydroxyl group from 2-methylestrone. This compound is an intermediate in the preparation of 2-methylestratrienes (see under Synthetic Methods) and was also ex-

(11) The term lipodiatic was suggested by Dr. H. W. Sause of the Division of Chemical Research, G. D. Searle and Co. It is derived from the two Greek words, lipos (fat) and diaito (regulate). In man the lipodiatic action of an estrogen usually includes a reduction in plasma cholesterol, cholesterol-phospholipid ratios, and β -lipoproteins and an increase in α -lipoproteins. Such alterations in plasma lipids should be beneficial if the lipid composition of plasma exerts an influence on the development of atherosclerosis.



aminated pharmacologically. The three values (lipodiatic, estrogenic, and their ratio) corresponding to Table I are 28%, 1.2%, and 23 for 2-methylestrone. If removal of the phenolic hydroxyl has an effect of the same order of magnitude as for estrone, then the values for 2-methylestratrien-17-one would be approximately 30%, 0.1%, and 300. The lower lipodiatic and estrogenic indices in Table I (<5 and <0.01, respectively) suggest that the 2-methyl group has an effect additional to its effect in 3-oxygenated structures, possibly steric interference with biological reoxidation at position 3.

Synthetic Methods.—In the preparation of estratrienes unsubstituted in the A-ring, the 3-oxygen atom of either estrone or estradiol was removed by reduction of the diethyl phosphate ester with lithium in liquid ammonia.¹² Estradiol proved to be more convenient than estrone for this sequence, since the latter led to only partial reduction of the 17-keto function.

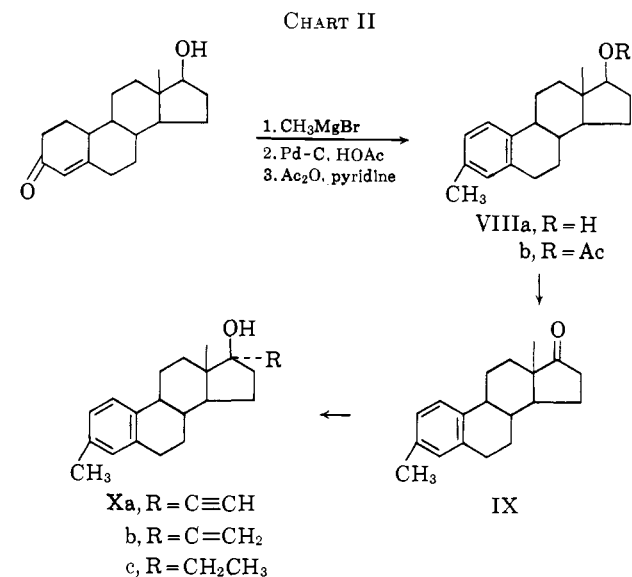
Preparation of the phosphate ester was carried out by reaction of the appropriate phenoxide anion with diethyl chlorophosphate, shown in Chart I, to give the

(12) G. W. Kenner and N. R. Williams, *J. Chem. Soc.*, 522 (1955).

esters Ia and Ib which were isolated and characterized. This method of preparation was more successful in these cases than the method of generating diethyl chlorophosphate *in situ*.¹² Subsequent removal of the ester group and acetylation or oxidation gave II and III^{2,13} whose syntheses by other methods have been described.¹³

Reduction of the diethyl phosphate ester of 1-hydroxy-4-methylestra-1,3,5(10)-trien-17 β -ol (V) and the dienol-benzene rearrangement¹⁴ of 1,4-androstadiene-3,17 β -diol provided useful routes to the 4-methylestratrienes VIa-c.^{14a,15}

3-Methylestratrienes were prepared from 19-nortestosterone¹⁶ by the route shown below (Chart II). Purification of the alcohol VIIIa was accomplished through



of the alcohol VIIIa was accomplished through its acetate which was isolated by direct crystallization.

The ketones IX, III, and VIc (Chart I) served as starting materials for preparation of the various 17-substituted derivatives, Xa-c, IVa-d, and VIIa-d, respectively.

Finally, it was desired to prepare some 2-methylestratrienes since this series was of interest for both pharmacological and chemical comparisons. The latter interest arose in recognition of the possibility of Wagner-Meerwein-type shifts in the preparation of VIII. Ultraviolet spectral properties of 1,2,3- and 1,2,4-trialkylated benzenes are significantly different¹⁷ and the data for VIII require assignment of the 2- or 3-position to the methyl group. Though position 2 was an unlikely possibility under the conditions of preparation and isolation of VIIIb, comparison with an authentic 2-methyl isomer was indicated. The series was entered by removal of the 3-oxygen, through the phosphate ester as for II and VI, from 2-methylestradiol.¹⁸

(13) (a) E. Caspl, E. Cullen, and P. K. Grover, *ibid.*, 212 (1963); (b) E. Hecker, *Chem. Ber.*, **95**, 977 (1962).

(14) (a) M. J. Gentles, J. B. Moss, H. L. Herzog, and E. B. Hershberg, *J. Am. Chem. Soc.*, **80**, 3702 (1958); (b) H. Dannenberg and H. Neumann, *Ann. Chem.*, **646**, 148 (1961).

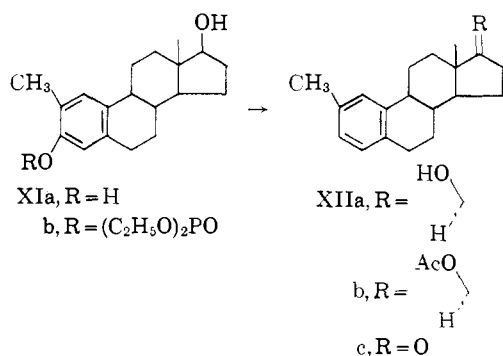
(15) H. Dannenberg and C. H. Doering, *Z. Physiol. Chem.*, **311**, 84 (1958).

(16) A. J. Birch, *J. Chem. Soc.*, 367 (1950).

(17) "Organic Electronic Spectral Data," M. J. Kamlet, Ed., Interscience Publishers, Inc., New York, N. Y., 1960; for example, see the 1,2,3- and 1,2,4-trimethylbenzenes or the 5- and 6-methyltetralins.

(18) (a) T. L. Patton, *J. Org. Chem.*, **25**, 2148 (1960); (b) J. Iriarte and H. J. Ringold, *Tetrahedron*, **3**, 28 (1958); (c) D. H. Peterson, L. M. Reineke, H. C. Murray, and O. K. Sebek, *Chem. Ind. (London)*, 1301 (1950).

Acetylation and oxidation gave XIIb and c which were shown to be different from VIIIb and IX by melting point behavior and by comparison of infrared and n.m.r. spectra.¹⁹



Experimental²⁰

Estrone Diethylphosphate (Ib).²¹—To 4.6 g. (3.4% excess) of sodium hydroxide in 20 ml. of water under nitrogen was added 30 g. of estrone and 120 ml. of ethanol. The mixture was stirred until the estrone dissolved and was then cooled in an ice bath. Diethyl chlorophosphite²² (22.4 g., 17% excess) was added dropwise to the stirred, ice-cold solution over a period of 20 min. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 3 hr.

The reaction mixture was partitioned between ether (200 ml.) and water (50 ml.), and the layers were separated. The ether solution was extracted three times with water and dried (Na_2SO_4), and the ether was removed to give 44 g. of a colorless oil which crystallized on standing. Chromatography of 1.4 g. of this material on 35 g. of silica gel in benzene and benzene-ethyl acetate mixtures gave a small amount of estrone in 10% ethyl acetate-benzene. Further elution with 10, 15, and 20% ethyl acetate-benzene gave 730 mg. of the phosphate ester. Recrystallization from ether-petroleum ether (b.p. 60–71°) gave 500 mg. of pure estrone diethyl phosphate (Ib): m.p. 67–68°; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 5.75, 6.22, 6.32, 7.85, 9.0–9.2, and 10.15–10.3 μ ; λ_{max} 269 m μ (ϵ 987) and 276.5 m μ (ϵ 913); $[\alpha]_{\text{D}} +110.3^\circ$.

Anal. Calcd. for $\text{C}_{22}\text{H}_{31}\text{O}_5\text{P}$: C, 65.01; H, 7.69. Found: C, 64.64; H, 7.36.

Estradiol 3-Diethylphosphate (Ia).^{21,23} **A.**—To an ice-cold solution of 1.0 g. of estrone 3-diethylphosphate (Ib) in 5 ml. of ethanol was added a solution of 0.5 g. of NaBH_4 in 2 ml. of water and 3 ml. of ethanol. After 5 min., the reaction mixture was poured into ether and extracted three times with water. After drying and concentration of the ether solution, crystallization occurred to give 340 mg. of product: m.p. 113.5–115.5°; $\lambda_{\text{max}}^{\text{KBr}}$ 2.88, 6.2, 6.3 μ ; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 7.88 and 9.64 μ (both broad); $[\alpha]_{\text{D}} +57.0^\circ$.

Anal. Calcd. for $\text{C}_{22}\text{H}_{33}\text{O}_5\text{P}$: C, 64.69; H, 8.14. Found: C, 64.76; H, 8.11.

A second crop of 400 mg., m.p. 112.0–113.5°, was obtained from the filtrate. Its infrared spectrum was identical with that of the first crop.

B.—To 30 g. of estradiol suspended in 50 ml. of ethanol under nitrogen was added 13.2 ml. of 8.5 N KOH with stirring. The resulting solution was cooled in an ice bath and 21.85 g. of

diethyl chlorophosphite was added in approximately 5 min. with stirring. After an additional 5 min. the reaction mixture (pH ca. 7) was poured into ether and extracted three times with water. The ether solution was dried, and the solvent was removed to give 50 g. of a light amber oil. Its infrared spectrum was essentially identical with that of the material obtained by the borohydride reduction (A above). This material was used directly in the following experiment.

Estra-1,3,5(10)-trien-17 β -ol (IIa).²³—Fifty grams of estradiol 3-diethyl phosphate (Ia) in 50 ml. of ether was diluted to approximately 300 ml. with liquid ammonia. Portionwise addition of lithium to the reaction mixture while stirring required 2 equiv. of lithium (1.7 g.) to produce a transient blue coloration. The NH_3 was allowed to evaporate, and the residue in ether was extracted twice with water, twice with 10% H_2SO_4 , twice with Claisen's alkali, and finally twice with water. The ether layer was dried, and the ether was removed to give 22 g. (78%) of crude product. Recrystallization from ether-petroleum ether (b.p. 28–38°) gave 15.0 g., m.p. 115–117.5° (lit.¹³: 109–110°). An analytically pure sample of m.p. 117–118.5° had an essentially identical infrared spectrum (KBr pellet): $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.72, 7.16, 7.27, 7.46 μ ; λ_{max} 266 m μ (ϵ 501) and 273.5 m μ (ϵ 514); $[\alpha]_{\text{D}} +89.7^\circ$.

Anal. Calcd. for $\text{C}_{26}\text{H}_{42}\text{O}$: C, 84.32; H, 9.44. Found: C, 84.14; H, 9.32.

17 β -Acetoxyestra-1,3,5(10)-triene (IIb).—Estra-1,3,5(10)-trien-17 β -ol (IIa, 590 mg.) was heated with 3 ml. of pyridine and 2 ml. of acetic anhydride on the steam bath for 1 hr. The reaction mixture was cooled and diluted with water to give 665 mg. of crude product. Recrystallization from methanol gave 610 mg. of 17 β -acetoxyestra-1,3,5(10)-triene (IIb): m.p. 122–124°; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 5.76 and 7.9 μ ; λ_{max} 266 m μ (ϵ 527) and 273.5 m μ (ϵ 516); $[\alpha]_{\text{D}} +51.4^\circ$.

Anal. Calcd. for $\text{C}_{26}\text{H}_{40}\text{O}_2$: C, 80.49; H, 8.78. Found: C, 80.39; H, 9.28.

Estra-1,3,5(10)-trien-17-one (III).²³ **A.**—To an ice-cold solution of 2.24 g. estra-1,3,5(10)-trien-17 β -ol (IIa) in 20 ml. of acetone was added 2.3 ml. (5% excess) of 8 N chromium trioxide in 8 N H_2SO_4 ,^{24,25} dropwise with stirring. After 4 min., dilution with water gave III which was collected, washed with water and cold methanol, and dried to give 2.02 g., m.p. 136–139° (lit.¹³: 135–136°). The infrared spectrum was identical with that of material obtained in B.

B.—Estra-1,3,5(10)-trien-17 β -ol (IIa, 350 mg.) in 4 ml. of acetic acid was treated with a solution of chromium trioxide in 2 ml. of water. After stirring for 45 min., dilution with water gave 340 mg. of product, m.p. 133–138°. Recrystallization from ether-petroleum ether (b.p. 28–38°) gave 270 mg. III as transparent prisms: m.p. 137–142°; $\lambda_{\text{max}}^{\text{KBr}}$ 5.75 μ ; λ_{max} 266 m μ (ϵ 533) and 273 m μ (ϵ 504); $[\alpha]_{\text{D}} +163.0^\circ$.

Anal. Calcd. for $\text{C}_{26}\text{H}_{42}\text{O}$: C, 84.99; H, 8.72. Found: C, 85.02; H, 8.27.

17 α -Ethyneestra-1,3,5(10)-trien-17 β -ol (IVa).²³—Potassium (4 g.) was dissolved in approximately 50 ml. of *t*-amyl alcohol by heating in a nitrogen atmosphere. Excess solvent was removed from the hot solution in a stream of nitrogen until potassium *t*-amylate began to crystallize from the solution at reflux. After cooling with stirring, 15 ml. of anhydrous ether was added, and the cold mixture was saturated with acetylene with rapid stirring. Then 3.0 g. of estra-1,3,5(10)-trien-17-one was added and a slow stream of acetylene was passed through the cold reaction mixture for 7.5 hr. After 16 hr. at 0–5°, saturated NH_4Cl solution was added, and the product was isolated in ether after extraction with NH_4Cl solution, 5% HCl, and water. The ether was removed, and a benzene solution of the residue was chromatographed over 300 g. of silica gel (60–200 mesh). Elution with benzene gave fractions of the desired product with melting points in the range of 120–125°. Recrystallization from ether-petroleum ether (b.p. 60–71°) gave 2.13 g. of IVa: m.p. 123.5–125.5°; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.75 and 3.02 μ ; λ_{max} 265.8 m μ (ϵ 512) and 272.8 m μ (ϵ 514); $[\alpha]_{\text{D}} 8.48^\circ$.

Anal. Calcd. for $\text{C}_{26}\text{H}_{42}\text{O}$: C, 85.07; H, 8.63. Found: C, 85.74; H, 8.78.

17 α -Ethylestra-1,3,5(10)-trien-17 β -ol (IVc).—17 α -Ethyneestra-1,3,5(10)-trien-17 β -ol (IVa, 610 mg., in 15 ml. of ethanol) was reduced at atmospheric pressure and room temperature in

(19) In the latter, there is a distinct difference in the splitting pattern for the aromatic protons of corresponding 2- and 3-methyl-3-desoxyestratrienes. This appears in the apparent multiplicity and resolution of bands.

(20) All ultraviolet spectra were run in methanol and rotations were taken in chloroform unless otherwise indicated. Chemical shifts of the n.m.r. bands were determined in deuteriochloroform and are expressed as δ , or parts per million less than the field required for tetramethylsilane resonance when determined as an internal standard. The authors wish to thank Dr. R. T. Dillon of the analytical division of G. D. Searle and Co. for the analytical and optical data reported and Dr. E. G. Daskalakis and staff for the paper and column chromatography.

(21) K. Sakakibara, M. Sawai, and I. Chuman, U. S. Patent 3,081,316 (March 12, 1963).

(22) G. M. Steinberg, *J. Org. Chem.*, **15**, 637 (1950). We wish to express our appreciation to Mr. James M. Schlatter for preparation of generous quantities of this reagent.

(23) A. H. Goldkamp, U. S. Patent 2,947,763 (August 2, 1960).

(24) F. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(25) K. Bowden, I. M. Hedden, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

the presence of 100 mg. of 10% palladium on carbon (uptake, 102.6 ml.; theory, 100 ml.). The catalyst was filtered off, and the filtrate was diluted with approximately 1 vol. of water to give 240 mg. of IVc: m.p. 109.5–111.5°; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.74 and 6.7 μ ; λ_{max} 265.8 (ϵ 486) and 273 (ϵ 488); $[\alpha]_D +57.9^\circ$; $\delta = 0.88$, 1.0, 1.11 (C-21), 0.91 (C-18), 2.8–3.0 (C-6), 7.08, 7.12, 7.23 (C-1 to C-4), and 1.20 (O–H, removed on D₂O exchange) p.p.m.

Anal. Calcd. for C₂₀H₂₈O: C, 84.45; H, 9.92. Found: C, 84.62; H, 10.00.

17 α -Vinylestra-1,3,5(10)-trien-17 β -ol (IVb).²³—A solution of 1.16 g. of 17 α -ethynylestra-1,3,5(10)-trien-17 β -ol (IVa) of 80% purity (ca. 20% 17-ketone) in 14 ml. of pyridine was treated with hydrogen and 200 mg. of 5% Pd–CaCO₃.²⁶ After an uptake of 75 ml. of hydrogen (theory 81 ml.), the reaction mixture was filtered, and the solvent was removed. Chromatography over 100 g. of silica gel gave fractions of the desired product totaling 400 mg. (m.p. range 103–107°) on elution with 75% benzene–petroleum ether (b.p. 60–71°). Recrystallization from petroleum ether gave a mixture of prisms and matted needles. The latter melted at 75–80° and resolidified in prisms; the total melting point sample melted at 105–109°. Repeated recrystallization with slow cooling gave 129 mg. of prisms: m.p. 109–111°; m.m.p. 99–109° with IVc; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.75 and 6.7 μ ; λ_{max} 266 m μ (ϵ 522) and 283.5 m μ (ϵ 452); $[\alpha]_D +58.8^\circ$; $\delta = 0.95$ (C-18), 2.8–3.0 (C-6), 5.04, 5.08, 5.25, 5.33 (C-21, 2 protons), 5.92, 6.10, 6.21, 6.38 (C-20, 1 proton), 7.08, 7.12, 7.23 (C-1 to C-4) p.p.m.

Anal. Calcd. for C₂₀H₂₈O: C, 85.02; H, 9.29. Found: C, 85.16; H, 9.14.

17 α -Methylestra-1,3,5(10)-trien-17 β -ol (IVd).—An initial unsuccessful attempt to prepare this material from 600 mg. of the 17-ketone using methylolithium in tetrahydrofuran gave, after chromatography, 200 mg. of a mixture containing approximately 20% of the starting ketone. This was combined with 210 mg. of the ketone and re-treated with 10 ml. of 3 *M* ethereal methylmagnesium bromide and 15 ml. of benzene at reflux. After 5.5 hr., the reaction was quenched with water, benzene was added, and the benzene layer was extracted with water and saturated NH₄Cl solution. The benzene solution was dried, solvent was removed, and the residue (450 mg.) was crystallized from petroleum ether (b.p. 28–38°) to give 110 mg. of IVd as fine, matted needles: m.p. 108.5–111.5°; $\lambda_{\text{max}}^{\text{KBr}}$ 2.98, 3.4, 3.48, 3.53, 6.7, 7.27, and 13.43 μ .

Anal. Calcd. for C₁₉H₂₆O: C, 84.39; H, 9.69. Found: C, 84.67; H, 9.81.

The residue from the filtrate was repeatedly recrystallized from ether–petroleum ether (b.p. 28–38°) to give an additional 39 mg. of product, m.p. 108–112°; the infrared spectrum was identical with that of the first crop.

4-Methylestra-1,3,5(10)-trien-17 β -ol (VIa).—This compound was prepared at various times by (a) the lithium in liquid ammonia reduction¹² of the diethyl phosphate ester of 1-hydroxy-4-methylestra-1,3,5(10)-trien-17-one,²⁷ in 42% yield over-all; (b) the dienol–benzene rearrangement of 1,4-androstadiene-3,17 β -diol,^{14a} in 54% yield; and (c) the NaBH₄ reduction of 4-methylestra-1,3,5(10)-trien-17-one^{13a} (VIc), in 96% yield. The compound crystallized from cyclohexane as colorless needles, m.p. 115–116.5°, $[\alpha]_D +63.0^\circ$ (lit.¹⁵ m.p. 114–116°, $[\alpha]_D +66^\circ$).

4-Methylestra-1,3,5(10)-trien-17 β -ol Acetate (VIb).—Acetylation of 4-methylestra-1,3,5(10)-trien-17 β -ol (VIa) with 1:1 acetic anhydride–pyridine on the steam bath for 0.5 hr. gave the acetate in 86% yield as colorless prisms (from ethyl acetate–ethanol), m.p. 178.5–181.5° (lit.¹⁵ m.p. 174–176°), $[\alpha]_D +34.4^\circ$.

4-Methylestra-1,3,5(10)-trien-17-one (VIc).—This compound was prepared both by the chromic acid oxidation of 4-methylestra-1,3,5(10)-trien-17 β -ol (VIa) in 86% yield, and by the dienol–benzene rearrangement of 17-ethylenedioxy-1,4-androstadien-3-one^{14a} in 58% yield. The compound crystallized from ethyl acetate–ethanol as colorless prisms, m.p. 183.5–185°, $[\alpha]_D +143.5^\circ$ (lit.^{14a} m.p. 184.5–186°, $[\alpha]_D +146^\circ$).

17 α -Ethynyl-4-methylestra-1,3,5(10)-trien-17 β -ol (VIIa).—Ethynylation of 4-methylestra-1,3,5(10)-trien-17-one (VIc, 5.37 g., 0.02 mole) was carried out by a procedure analogous to one used by Colton, *et al.*²⁸ The crude product was chromatographed over 540 g. of silica gel. Elution of the column with benzene gave 3.16 g. of VIIa in fractions melting in the range 135.5–139°.

Recrystallization from petroleum ether (b.p. 60–70°) gave fine colorless needles, m.p. 138–139°, $[\alpha]_D -4.0^\circ$, λ_{max} 263 m μ (ϵ 235).

Anal. Calcd. for C₂₁H₂₆O: C, 85.66; H, 8.90. Found: C, 85.91; H, 8.80.

17 α -Allyl-4-methylestra-1,3,5(10)-trien-17 β -ol (VIIb).—4-Methylestra-1,3,5(10)-trien-17-one (VIc, 2.15 g., 8 mmoles) was treated with the Grignard reagent prepared from 10.0 g. of allyl bromide after the manner of Colton, *et al.*²⁸ Direct crystallization of the crude product from methanol gave 1.49 g. of fine colorless needles of VIIb, m.p. 115–116°, $[\alpha]_D +71.2^\circ$, λ_{max} 263 m μ (ϵ 235).

Anal. Calcd. for C₂₂H₃₀O: C, 85.11; H, 9.74. Found: C, 84.81; H, 9.90.

4,17 α -Dimethylestra-1,3,5(10)-trien-17 β -ol (VIIc).—A solution of 1.07 g. (4 mmoles) of 4-methylestra-1,3,5(10)-trien-17-one (VIc) in 50 ml. of tetrahydrofuran was added to 15 ml. of 3 *M* ethereal methylmagnesium bromide in 50 ml. of tetrahydrofuran. The mixture was heated under reflux for 3 hr. and then worked up using saturated NH₄Cl solution. The crude product was chromatographed over 85 g. of silica gel. Elution of the column with 5% ethyl acetate in benzene gave 72 mg. of VIIc followed by 0.795 g. of VIIc in fractions melting in the range 150–160°. Recrystallization of the latter from methanol gave colorless needles, m.p. 162–163°, $[\alpha]_D +46.0^\circ$, λ_{max} 263.5 m μ (ϵ 244).

Anal. Calcd. for C₂₀H₂₈O: C, 84.45; H, 9.92. Found: C, 84.51; H, 9.87.

17 α -Ethyl-4-methylestra-1,3,5(10)-trien-17 β -ol (VIIId).—17 α -Ethynylestra-1,3,5(10)-trien-17 β -ol (VIIa, 1.03 g., 3.5 mmoles) in ethanol was reduced at atmospheric pressure and room temperature in the presence of 5% palladium on carbon (uptake: 109% yield). The catalyst was filtered off, and the filtrate was evaporated to dryness. Recrystallization from methanol gave 0.75 g. of fine colorless needles of VIIId melting, after being dried *in vacuo* at 64° overnight, at 114.5–115°, $[\alpha]_D +37.1^\circ$, λ_{max} 263 m μ (ϵ 245).

Anal. Calcd. for C₂₁H₃₀O: C, 84.51; H, 10.13. Found: C, 84.17; H, 9.90.

17 β -Acetoxy-3-methylestra-1,3,5(10)-triene (VIIIb).²³—Sixty grams of 17 β -hydroxy-19-norandrost-4-en-3-one was dissolved in a mixture of tetrahydrofuran (40 ml.) and ether containing sufficient CH₂Cl₂ to dissolve the solid. The solution (800 ml.) was boiled down to 600 ml. to remove most of the methylene chloride. This was added over 1.5 hr. to a refluxing, 3 *M* ethereal methylmagnesium bromide solution with rapid stirring. After an additional 3 hr., the reaction mixture was cooled and extracted three times with saturated NH₄Cl solution. The ether layer was dried, and the solvent was removed to give 64 g. of residue. The infrared spectrum showed 5–10% of unreacted ketone (5.98 μ). Glacial acetic acid (500 ml.) and 20 g. of 10% palladium on charcoal were added, and the reaction mixture was stirred and brought rapidly to reflux. After 30 min. the mixture was cooled and filtered and the acetic acid was removed by distillation under reduced pressure. The residue was treated with 100 ml. of pyridine and 75 ml. of acetic anhydride for 2 hr. on the steam bath. After treatment with Darco, addition of ice and water gave 60 g. of material. Two recrystallizations from methanol and one from acetone–methanol gave 14.07 g. of VIIIb, m.p. 125.5–128°. Chromatography of the residue from the combined filtrates over silica gel gave an additional 9.5 g. of VIIIb on elution with 60% benzene–petroleum ether (b.p. 60–71°). Recrystallization from methylene chloride–methanol gave 8.5 g. of VIIIb. The melting point and infrared spectrum were identical with that of the material obtained above; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 5.77 and 7.9 μ ; λ_{max} 269 m μ (ϵ 718) and 278 m μ (ϵ 804); $[\alpha]_D +53.0^\circ$.

Anal. Calcd. for C₂₁H₃₂O₂: C, 80.73; H, 9.03. Found: C, 80.29; H, 8.67.

A mixture melting point with an authentic sample of the 1-methyl isomer of m.p. 126.5–127.5°²⁹ was 108.5–113.5°; with the 2-methyl isomer, 98.5–121°.

3-Methylestra-1,3,5(10)-trien-17 β -ol (VIIIa).—17 β -Acetoxy-3-methylestra-1,3,5(10)-triene (VIIIb, 300 mg.) in 10 ml. of methanol and 3 ml. of 8.5 *N* aqueous KOH was heated under reflux for 75 min. The reaction mixture was poured into ether and, after two extractions with water, the ether solution was dried and the

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(29) The authors wish to thank Professor H. Dannenberg of the Max-Planck Institut für Biochemie for authentic samples of 17 β -acetoxy-1-methyl- and 17 β -acetoxy-4-methylestratrienes for comparison (see ref. 14b).

solvent was removed to give 240 mg. of an oil. Crystals (m.p. 46–52°) could be obtained from aqueous methanol or methanol (m.p. 61–70°) with considerable loss of material. The oil had an infrared spectrum identical with that of the crystalline material which, after drying under reduced pressure for 24 hr. at 40°, gave an analysis corresponding to a hemisolvate: $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.75, 2.9, 6.2, 7.11, 7.22, 7.43 μ ; λ_{max} 269 $\text{m}\mu$ (ϵ 730) and 278 $\text{m}\mu$ (ϵ 811).

Anal. Calcd. for $\text{C}_{19}\text{H}_{26}\text{O} \cdot 0.5\text{H}_2\text{O}$: C, 81.67; H, 9.74. Calcd. for $\text{C}_{19}\text{H}_{26}\text{O} \cdot 0.5\text{CH}_3\text{OH}$: C, 81.77; H, 9.85. Found: C, 81.70; H, 9.47.

3-Methylestra-1,3,5(10)-trien-17-one (IX).—Chromium trioxide reagent^{24,25} (0.352 ml.) was added dropwise with stirring to a solution of 380 mg. of 3-methylestra-1,3,5(10)-trien-17 β -ol (VIIIa) in 4 ml. of acetone. Dilution with water gave crystalline material which was twice recrystallized from methanol to give 156 mg. of IX: m.p. 174–181°; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 5.74 and 7.25 μ ; λ_{max} 269 $\text{m}\mu$ (ϵ 751) and 278 $\text{m}\mu$ (ϵ 829). Paper chromatography did not show any impurity; $\delta = 0.89$ (C-18, 3 protons), 2.28 (3-methyl), 2.7–3.05 (C-6), 6.95, 7.04, 7.16, 7.28 (A-H, 3 protons) p.p.m.

Anal. Calcd. for $\text{C}_{19}\text{H}_{26}\text{O}$: C, 85.02; H, 9.01. Found: C, 85.01; H, 9.19.

Isolation of the product in benzene after extraction with dilute NaHCO_3 and dilute HCl gave material of slightly higher melting point (179–182.5°) after recrystallization. The infrared spectrum was identical with the material described above: $[\alpha]_D^{25} +161.4^\circ$.

17 α -Ethylnyl-3-methylestra-1,3,5(10)-trien-17 β -ol (Xa).—The reaction was carried out using IX with conditions as described for ethynylation of III. The crude product from 2.95 g. of the 17-ketone was chromatographed over 300 g. of silica gel in benzene. This gave fractions melting in the range of 121–123°. Recrystallization from methanol–water gave 2.4 g. of Xa: m.p. 123.5–125.5°; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.76 and 3.02 μ ; λ_{max} 269 $\text{m}\mu$ (ϵ 704) and 278 $\text{m}\mu$ (ϵ 809); $[\alpha]_D^{25} +5.5^\circ$.

Anal. Calcd. for $\text{C}_{21}\text{H}_{28}\text{O}$: C, 85.66; H, 8.90. Found: C, 85.32; H, 8.77.

17 α -Vinyl-3-methylestra-1,3,5(10)-trien-17 β -ol (Xb).—Reduction of 700 mg. of Xa was carried out as described above for the reduction of IVa to IVb. The hydrogen uptake was 58 ml. (calcd., 56.1 ml.). Direct crystallization from aqueous methanol gave 600 mg. of Xb: m.p. 95–96°; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.75 and 10.8 μ ; λ_{max} 269 $\text{m}\mu$ (ϵ 718) and 277.8 $\text{m}\mu$ (ϵ 814); $[\alpha]_D^{25} +57.4^\circ$; δ same as IVb for C-18, C-6, C-20, and C-21 plus 2.29 (C-3 methyl, 3 protons) and 6.88, 7.01, 7.12, 7.23 (A–H, 3 protons) p.p.m.

Anal. Calcd. for $\text{C}_{20}\text{H}_{28}\text{O}$: C, 85.98; H, 9.52. Found: C, 85.29; H, 9.57.

17 α -Ethyl-3-methylestra-1,3,5(10)-trien-17 β -ol (Xc).—Reduction of 700 mg. of 17-ethynyl-3-methylestra-1,3,5(10)-trien-17 β -ol (Xa) in ethanol in the presence of 5% palladium on charcoal required 114.4 ml. of hydrogen (calcd., 112.4 ml.). After crystallization by dilution with water, the product was air dried and then dried under reduced pressure at 57 and 78° to give 570 mg.: m.p. 96–97° (gradual change of crystal form above 60°; it melted immediately when placed on the block at 80°, then resolidified); $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.74, 6.2, 6.65, 6.82, 7.24, 10.27 μ ; $[\alpha]_D^{25} +51.4^\circ$; δ C-18, C-21, and C-6 as in IVc plus 2.28 (C-3 methyl, 3 protons) and 6.89, 7.0, 7.13, 7.23 (A–H, 3 protons) p.p.m.

Anal. Calcd. for $\text{C}_{21}\text{H}_{30}\text{O}$: C, 84.51; H, 10.31. Found: C, 84.58; H, 9.96.

2-Methylestrone.—This compound was prepared by the method of Patton^{18a} and had m.p. 226.5–233° (lit.¹⁸ 233, 221–225, and 243–244°); $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.75, 5.73, 7.26 μ ; λ_{max} 280–285 $\text{m}\mu$ (ϵ 2645) [lit.^{18b,c} 283 $\text{m}\mu$ (ϵ 2565, 2650)].

Anal. Calcd. for $\text{C}_{19}\text{H}_{26}\text{O}_2$: C, 80.24; H, 8.51. Found: C, 79.99; H, 8.54.

2-Methylestradiol (XIa).—2-Methylestrone (5.71 g.) was dissolved in 50 ml. of tetrahydrofuran and 50 ml. of ethanol by warming. The solution was cooled to room temperature and a solution of 7 g. NaBH_4 in 25 ml. of water was prepared and added with swirling. After 10 min., the reaction mixture was poured onto ice, water was added, and the solid was collected. After air drying, it weighed 6.0 g.; m.p. 168.5–171.5° (lit.¹⁸ 182–183, 185–186, and 184–186°); $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.74 and 7.23 μ .

2-Methylestradiol 3-Diethylphosphate Ester (XIb).—2-Methylestradiol (5.94 g.) was suspended in 10 ml. of ethanol and dissolved by addition of 2.64 ml. of 8.5 *N* KOH with stirring. After cooling in an ice bath, 4.43 g. of diethyl chlorophosphate was added over 5 min. and stirring was continued for an additional 5 min. The reaction mixture was poured into ether and extracted three times with water. The ether solution was dried and the solvent was removed to give 7.42 g. of a viscous paste; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.74, 7.88 (broad), and 9.6 μ (broad).

2-Methylestra-1,3,5(10)-trien-17 β -ol (XIHa). The above phosphate ester (XIa, 7.4 g.) was stirred with ca. 50 ml. of NH_4OH while 0.25 g. of lithium was added portionwise over 1 hr. Most of the ammonia had evaporated to leave a paste which was solubilized by addition of 25 ml. of ether and 50 ml. of ammonia. Additional lithium (0.33 g.) was added over 1 hr. (a transient blue color was produced), and the ammonia and ether were removed. The residue was transferred with ether and dilute H_2SO_4 . After shaking, the layers were separated and the ether layer was extracted twice with 4 *N* NaOH and twice with water. Drying and removal of the ether gave 3.3 g. of crude XIHa, which solidified on standing; m.p. ca. 101–120° (opaque melt); $\lambda_{\text{max}}^{\text{CHCl}_3}$ 2.73, 6.2, and 7.23 μ ; λ_{max} 272.5 $\text{m}\mu$ (ϵ 943), 278 (1275), and 282–289 (sh).

17 β -Acetoxy-2-methylestra-1,3,5(10)-triene (XIIf).—The crude alcohol (XIHa, 660 mg.) was treated with 3 ml. of pyridine and 3 ml. of acetic anhydride overnight. Dilution with water and cooling gave an oil which solidified on standing and was collected. It was chromatographed over 100 g. of neutral alumina in ether. From the first 0.5–1 l. there was obtained 410 mg. of product, m.p. 101–108°. Two recrystallizations from methanol gave 180 mg. of XIIf: m.p. 109.5–111.5°; $\lambda_{\text{max}}^{\text{CHCl}_3}$ 5.76, 7.27, 7.92, 9.62 μ ; λ_{max} 269 $\text{m}\mu$ (ϵ 757) and 278 $\text{m}\mu$ (ϵ 865); $\delta = 0.83, 2.05, 2.31, 4.6–4.9, 7.0,$ and 7.14 p.p.m.

Anal. Calcd. for $\text{C}_{20}\text{H}_{28}\text{O}_2$: C, 80.73; H, 9.03. Found: C, 80.83; H, 9.06.

Mixture melting points (lit.²⁶ 125°) with the 1-methyl isomer and the 3-methyl isomer were 95–108.5° and 98.5–121°, respectively.

2-Methylestra-1,3,5(10)-trien-17-one (XIIfc).—The crude alcohol (XIIfa, 1.5 g.) in acetone was oxidized with 1.5 ml. of chromium trioxide reagent (8 *N* in 8 *N* H_2SO_4);^{24,25} ice and water were added and the solid was collected (m.p. 104–116°, viscous melt). It was chromatographed over 100 g. of neutral alumina in ether to give material of m.p. 136–149°. One recrystallization from methanol raised the melting point to 149–151° and a second recrystallization gave 540 mg., m.p. 149.5–152.5° (fine needles which appeared to resolidify in plates as melting proceeded); $\lambda_{\text{max}}^{\text{CHCl}_3}$ 5.74 and 7.25 μ ; λ_{max} 269 $\text{m}\mu$ (ϵ 741) and 278 $\text{m}\mu$ (ϵ 833); $\delta = 0.90$ (C-18), 2.32 (2-methyl, 3 protons), 7.03 and 7.17 (A–H, 3 protons) p.p.m.

Anal. Calcd. for $\text{C}_{19}\text{H}_{26}\text{O}$: C, 85.02; H, 9.01. Found: C, 85.25; H, 8.97.