739. *Light-absorption and Chemical Properties of Mircestrol, the Œstrogenic Substance of* Pueraria mirifica.

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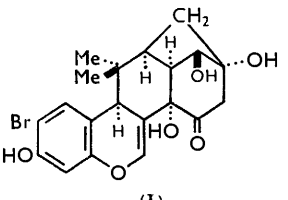
Mircestrol, isolated from *Pueraria mirifica* by a new method, has been converted into a monobromo-derivative, indicated by its molecular formula and light absorption to be a simple substitution product of the parent compound. *X*-Ray crystallographic analysis of this derivative by other authors [1] has given its complete structure (I), from which that of mircestrol (II) follows. The absorption spectra and some chemical properties of mircestrol are described in the light of this structure.

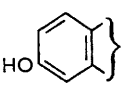
Various other derivatives of mircestrol have been prepared, including products of methylation, acetylation, and reduction by potassium borohydride. With bromine in methanol mircestrol yields a dibromo-methoxy-derivative, and with mineral acid gives an isomeric compound (isomircestrol); the structures (V) and (VIII; R = H) respectively are proposed for these products.

The presence of œstrogenic material in the tuberous roots of a woody, climbing plant found in northern Thailand was first reported in 1939,[2] after attention had been drawn to the fact that these roots were used locally as a rejuvenating drug. Later, the isolation from this source of a highly œstrogenic, pure substance was described,[3] and a preliminary chemical investigation of the compound was carried out.[4] The plant was at that time believed to be *Butea superba*, but has since been recognised as a new species and named *Pueraria mirifica* (Leguminoseae).[5] A new method has been developed [6] for the isolation in good yield, from the same source, of a potent œstrogen, believed to be identical with that previously described, and now named mircestrol. The œstrogenic activity of mircestrol in the mouse and the rat has been compared with that of œstradiol-17β and stilbœstrol,[6] and the activities of a number of the derivatives of mircestrol described here have also been determined.

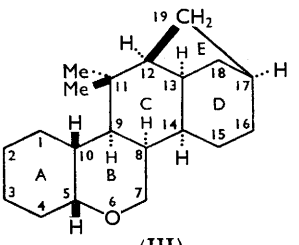
Our preliminary studies of the chemistry and light absorption of mircestrol indicated it to have a rather complex structure. Also, the amount available was small, and further plant material not readily obtainable, and so it seemed probable that it would be difficult to determine the structure by chemical means. An *X*-ray analysis was therefore carried out by Hodgkin, Rollett, and Taylor [1] on a monobromo-derivative of mircestrol prepared in these laboratories: they determined the complete structure (I) of this derivative, and the structure of mircestrol (II) follows.



(I)



(II)



(III)

We propose to name mircestrol and related compounds systematically as derivatives of the hypothetical compound (III) (with the relative stereochemistry shown), termed

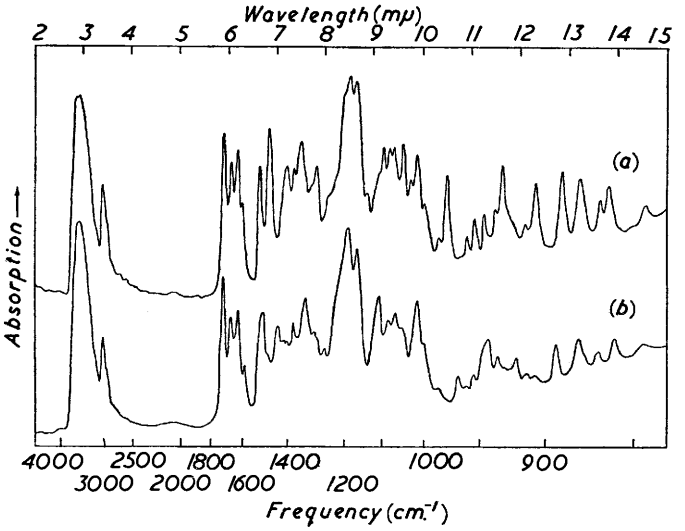
[1] Taylor, Hodgkin, and Rollett, *J.*, 1960, 3685.
[2] Vatna, *Thai Sci. Bull.*, 1939, No. 4, p. 3.
[3] Schoeller, Dohrn, and Hohlweg, *Naturwiss.*, 1940, **28**, 532.
[4] Butenandt, *Naturwiss.*, 1940, **28**, 533.
[5] Kashemsanta, Suvatabandhu, and Shaw, *Kew Bull.*, 1952, 549.
[6] Pope, Grundy, Jones, and Tait, *J. Endocrinol.*, 1958, **17**, xv.

mircestran; configurations relative to the 12,19-bond (beta) are denoted by the suffixes α and β.

The properties of mircestrol [3,14,17,18β-tetrahydroxymircestra-1,3,5(10),7-tetraen-15-one] are in good agreement with the known structure. Analyses and a molecular-weight determination indicate the molecular formula to be $C_{20}H_{22}O_6$, corresponding to (II). Active-hydrogen determinations confirm the presence of at least three hydroxyl groups, and the result of a Kuhn–Roth analysis is compatible with the presence of a *gem*-dimethyl group. The phenolic nature of the compound is shown by its solubility in aqueous potassium carbonate but not in potassium hydrogen carbonate, and by its reaction with diazotised amines to give dyes.

Treatment of mircestrol with methyl sulphate gave the alkali-insoluble 3-methyl ether in good yield. Diazomethane gave lower yields, owing to the ease of further reaction, affording mixtures which, unlike mircestrol and its 3-methyl ether, are soluble in non-polar

Infrared spectra of (*a*) *mircestrol* (II) *and* (*b*) *bromomircestrol* (I) *in* KBr *discs.*



solvents. One of these further products, a methyl ether of unknown constitution, has been isolated.

As expected from the presence of two tertiary hydroxyl groups, only two of the hydroxyl groups of mircestrol are readily acetylated, acetic anhydride (2·5 mol.) and pyridine under mild conditions giving a mixture of a mono- and a di-acetate. Although the infrared absorption of the former was not determined, it is believed to be the 3-acetate because, like the 3-methyl ether, it is very sparingly soluble in methanol. The infrared absorption spectrum of the diacetate includes bands at 1768 (phenolic acetate) and 1742 cm.$^{-1}$ (alcoholic acetate), making it probable that it is the 3,18β-derivative.

The infrared absorption of mircestrol (non-hygroscopic form; see p. 3701) due to O–H (3533, 3450 cm.$^{-1}$) is considerably stronger than that due to C–H (at 2950 cm.$^{-1}$), which is consistent with the presence of several hydroxyl groups. Carbonyl absorption occurs at 1706 cm.$^{-1}$, confirming the presence of an unconjugated keto-group in a six-membered ring. A moderately strong band occurs at 1661 cm.$^{-1}$, and this may be assigned to the –O–CH=C< group, for although unconjugated olefins generally absorb only weakly in this region, it has been reported [7] that attachment of an oxygen atom (of an acetoxy-group) to one of the olefinic carbon atoms considerably increases the intensity of the absorption with little effect on its frequency (cf. the Raman spectrum of chrom-2-en,

[7] Rosenkrantz and Gut, *Helv. Chim. Acta*, 1953, **36**, 1000.

which includes a strong band at 1669 cm.$^{-1}$ due to its ethylenic bond [8]). The remaining bands at frequencies above 1500 cm.$^{-1}$ are at 1623, 1597, and 1511 cm.$^{-1}$ and are evidently due to the benzene ring.

The strong absorption shown by mircestrol at 217 mμ (see Table) appears to be made up of the superimposed absorptions of the phenol ring and the group $-O-CH=C<$, since

Ultraviolet-light absorptions.

Compound *	λ (mμ)	ε	λ (mμ)	ε
Mircestrol (II)	217	21,650	285	4575
2-Bromomircestrol (I)	213	21,980	293	6895
Isomircestrol	232	13,640	273	4140
Isomircestrol methyl hemiacetal	232	14,770	275	3795
Dibromo-methoxy-derivative	221 †	11,000	288	6810
Potassium borohydride reduction product	230 †	7970	282	3670
Resorcinol monomethyl ether	222	6840	276	2145
			282	1885

* In 95% ethanol. † Inflection.

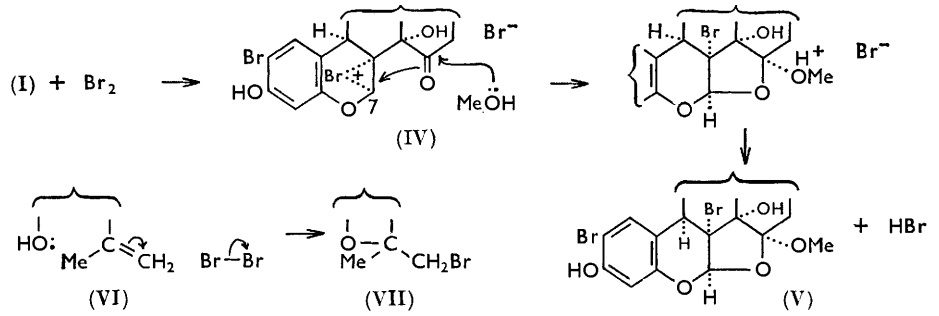
certain derivatives of mircestrol (the dibromo-methoxy-compound and potassium borohydride reduction product) which are phenols, but contain no $-O-CH=C<$ group, absorb similarly to resorcinol monomethyl ether in this region. The conclusion that the $-O-CH=C<$ chromophore absorbs at about 217 mμ is in agreement with the absorption maxima values reported [9] for several non-aromatic vinyl ethers; these have $\lambda_{max.}$ in the region of 205—210 mμ with ε *ca.* 7000. The values reported [10] for phenyl vinyl ether (in 96% ethanol) are $\lambda_{max.}$ 225 (ε 30,100) and 269 mμ (ε 1150).

Bromination of mircestrol was carried out in methanol, owing to the sparing solubility of the compound in non-hydroxylic solvents. The products were readily decomposed by dilute mineral acid and accordingly, on completion of a reaction, the hydrobromic acid present was at once neutralised with sodium hydrogen carbonate. Mircestrol with one mol. of bromine reacted incompletely, giving two main products, $C_{20}H_{21}O_6Br$ and $C_{20}H_{21}O_6Br_2 \cdot OMe$, both of which are phenolic. With 1·5 mol. of bromine reaction was virtually complete, the yields of the monobromo- and dibromo-methoxy-derivatives then being *ca.* 25% and 55% respectively. The former product is completely converted into the latter by the action of one mol. of bromine in methanol. As mentioned previously, *X*-ray analysis has shown the monobromo-derivative to have the constitution (I), and it is concluded from the absorption data that this compound differs structurally from mircestrol only in possessing a bromine atom in the aromatic ring. Their infrared spectra (see Figure) are similar even in the lower frequency region, and at higher frequencies the correspondence is close, the spectrum of the monobromo-compound including bands at 3380, 2935 (shoulder at *ca.* 2850), 1712 (C=O), 1664 ($-O-CH=C<$), and 1618, 1580, and 1499 (benzene ring) cm.$^{-1}$. The ultraviolet spectra (Table) are also very similar; in particular, both show the characteristic, strong absorption near 215 mμ.

The molecular formula of the dibromo-methoxy-derivative indicates that one molecule of bromine and one of methanol are involved in its formation from the monobromo-derivative. Its infrared absorption spectrum differs from that of the monobromo-derivative in that no bands are present at 1712 or 1664 cm.$^{-1}$, indicating loss of both the keto-group and the 7,8-double bond. The absence of this double bond is confirmed by the ultraviolet light absorption which at 213 mμ is much weaker than that of the monobromo-compound. The disappearance of the 7,8-double bond could be accounted for by the reaction: $-O-CH=C< + Br_2 + MeOH \longrightarrow -O-CH(OMe)-CBr< + HBr$, but this leaves the loss of the keto-group unexplained, and it seems possible that both groups might be involved in the reaction in the manner shown in formula (IV). The bromonium ion (IV) would be expected to be the initial product since the olefinic bond is strongly hindered

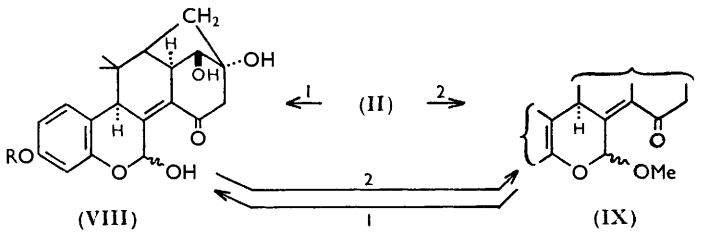
[8] Maitte, *Ann. Chim.* (*France*), 1954, **9**, 431.
[9] Eglinton, Jones, and Whiting, *J.*, 1952, 2873.
[10] Jacobs and Tuttle, *J. Amer. Chem. Soc.*, 1949, **71**, 1313.

on the front side of the molecule (particularly by the 11β-methyl group), but is much less so at the back. The hindrance at the front might then make it difficult for methanol to react with the ion (IV) in the normal way at $C_{(7)}$, but an indirect attack on this position through the keto-group as shown is indicated as possible by Stuart models; the dibromo-methoxy-derivative would consequently have structure (V). [A somewhat similar type



of mechanism (VI ⟶ VII) has been advanced [11] to explain the conversion of picrotoxinin nto α- and β-bromopicrotoxinin by aqueous bromine.] When the derivative had been treated with boiling, dilute hydrogen chloride in aqueous dioxan for 5 hr., paper chromatography of the products showed it to have reacted completely to give mainly a compound (which was phenolic) more soluble in polar solvents than the starting-material. This product did not crystallise, but the infrared spectrum of the crude material (determined in Nujol) included one (strong) band due to a carbonyl group, at 1711 cm.$^{-1}$. The appearance of this may be due to the formation of a 7-hydroxy-15-oxo-compound, through hydrolysis of the methyl hemiacetal group in (V), followed by opening of the lactol ring so formed. The dibromo-methoxy-compound, unlike mircestrol, yields no coloured materials with alkali in the presence of oxygen, and is only slowly attacked by hot, concentrated alkali in the absence of oxygen; it follows that neither bromine atom is readily displaced [in agreement with (V)], and also, that the saturation of the ethylenic bond and keto-group stabilises the molecule towards alkali.

Mircestrol, when warmed with concentrated hydrochloric acid in air, yields dark products after a short time. However, when the compound is heated in nitrogen with dilute hydrogen chloride in aqueous dioxan (or in dry dioxan, and then treated with aqueous sodium hydrogen carbonate) little coloured materials result, and there is formed in good yield a phenol (soluble in aqueous sodium hydroxide but not in sodium hydrogen carbonate, and yielding a dye with a diazotised amine solution), which is isomeric with mircestrol. This compound (isomircestrol) is indicated by paper chromatography of the total products



Reagents: 1, H_2O–HCl; 2, MeOH–HCl.

of the reaction to be formed irreversibly from mircestrol. The infrared absorption spectrum of isomircestrol at higher frequencies differs from that of mircestrol in including a band at 1678 cm.$^{-1}$ but not at 1706 or 1661 cm.$^{-1}$. The absence of absorption at *ca.* 1660 cm.$^{-1}$,

[11] Conroy, *J. Amer. Chem. Soc.*, 1957, **79**, 1726.

and the fact that the light absorption of isomircestrol is much weaker than that of mircestrol at 217 mμ, show that the group –O–CH=C< is not present in isomircestrol. Further, that the latter compound does not absorb at *ca.* 1706 cm.$^{-1}$ whilst it does so at 1678 cm.$^{-1}$, indicates that isomircestrol contains no unconjugated keto-group, but does contain an αβ-unsaturated keto-group. The presence of the latter is confirmed by the strong absorption of isomircestrol at 232 mμ. These differences in absorption can be accounted for if mircestrol with hot, dilute acid undergoes allylic re-arrangement; isomircestrol would then have structure (VIII; R = H). This conclusion is supported by the fact that isomircestrol is the only phenol which has been detected by paper chromatography in the total products of the reaction. [A similar acid-catalysed re-arrangement is that of Me_2C=CH·CMe(OH)·COMe to Me_2C(OH)·CH=CMe·COMe;[12] here, as expected, the equilibrium is strongly in favour of the conjugated ketone.] Mircestrol 3-methyl ether, when heated in dilute hydrochloric acid, appeared to re-arrange in the same way as mircestrol, giving an alkali-insoluble monomethyl ether with infrared absorption very similar to that of isomircestrol and therefore probably of structure (VIII; R = Me). The value calculated (259 mμ) by using Woodward's rules[13] for structure (VIII; R = H), differs considerably from the maximum observed (232 mμ) for isomircestrol; it is probable, however, that 232 mμ is not the true absorption maximum of this chromophore, owing to masking by the benzenoid absorption of isomircestrol.

As a cyclic hemiacetal (VIII; R = H), isomircestrol should be methylated by methanolic hydrogen chloride; this reagent does give an alkali-soluble methyl ether, with light absorption very similar to that of isomircestrol, and is reconvertible into the latter under acetal-hydrolysing conditions. This ether is therefore probably the methyl hemiacetal (IX; R = H). This compound is also formed by treatment of mircestrol with methanolic hydrogen chloride in the cold, an anionotropic change occurring similar to that by which isomircestrol is formed.

Zeisel analyses of mircestrol 3-methyl ether, isomircestrol methyl ether (VIII; R = Me), isomircestrol methyl hemiacetal, and the dibromo-methoxy-derivative gave unexpectedly high methoxyl values; this was accounted for when it was found that Zeisel analyses of mircestrol and isomircestrol also give positive values, although those compounds contain no alkoxy-groups. A number of non-alkoxy-, open-chain polyhydric alcohols have been reported[14] to give similar anomalous results.

Mircestrol, mircestrol 3-methyl ether, isomircestrol, and isomircestrol methyl hemiacetal with cold concentrated sodium hydroxide solution in air give dark solutions after 2—3 hr.; accordingly, reactions of these compounds in alkaline media have been carried out in nitrogen. Mircestrol with sodium hydroxide in nitrogen gives little coloured material unless the solution is heated; decomposition then occurs rather readily, paper chromatography indicating the main product to be a phenol less soluble than mircestrol in hydroxylic solvents.

Mircestrol and certain of its derivatives (see p. 3701), with a cold weakly acid, sodium periodate solution quickly give strong, yellow-green colours, whilst some other derivatives give similar colours on warming; the products generally fluoresce in ultraviolet light, and this has been of considerable value for the detection of these compounds on paper chromatograms.

Treatment of mircestrol with an excess of potassium borohydride gave, instead of the expected dihydro-derivative, a phenol which appears to have the formula $C_{20}H_{22}O_5$. Its light absorption differs from that of mircestrol in being much weaker at 217 mμ and in including no maxima at *ca.* 1706 and 1661 cm.$^{-1}$; the reaction therefore appears to involve loss of the 7,8-double bond as well as reduction of the carbonyl group of mircestrol.

[12] Braude and Timmons, *J.*, 1953, 3131.
[13] Woodward, *J. Amer. Chem. Soc.*, 1941, **63**, 1123; 1942, **64**, 76.
[14] von Rudloff, *Analyt. Chim. Acta*, 1957, **16**, 294; cf. Araki and Hasi, *J. Chem. Soc. Japan*, 1940, **61**, 99.

Fehling's solution, and also hot, alkaline solutions of triphenyltetrazolium bromide, are reduced by mircestrol, mircestrol 3-methyl ether, isomircestrol, and isomircestrol methyl hemiacetal. The non-ketonic dibromo-methoxy-derivative and potassium borohydride reduction product of mircestrol, however, do not reduce the latter reagent.

Experimental

M. p.s were determined on a Kofler block; because of the tendency to decomposition below the m. p.s, the temperature was raised as rapidly as possible to near the m. p. Infrared absorption spectra were measured for specimens pressed into potassium bromide discs. Kieselguhr used in chromatographic columns was "Celite 545." Hygroscopic compounds were desiccated in a "Quickfit" test-tube in an Abderhalden drying-pistol containing no chemical desiccant.

3,14,17,18β-*Tetrahydroxymircestra*-1,3,5(10),7-*tetraen*-15-*one* (*Mircestrol*) (II).—The *compound*, isolated from *Pueraria mirifica*,[6] crystallised from dry methanol as anhydrous, non-hygroscopic, rectangular plates, m. p. 268—270° (decomp.) both on a Kofler block and in a sealed capillary (Butenandt [4] reported m. p. *ca.* 260°), $[\alpha]_D^{17}$ +301° (*c* 1·08 in EtOH) (Found: C, 66·9, 67·15; H, 6·3, 6·4; *C*-Me, 3·0; active H, 0·75, 0·7. Calc. for $C_{20}H_{22}O_6$: C, 67·0; H, 6·2; 1*C*-Me, 7·6; 4H, 1·1%). Zeisel analysis gave an apparent 3·3% of methoxyl. Crystallisation of mircestrol from aqueous methanol gave mixtures (m. p. *ca.* 265°, decomp. >*ca.* 250°) of the anhydrous and a hydrated form (stout needles) in varying proportions. The hydrate is derived from a hygroscopic form since after desiccation at 60°/0·5 mm., the mixtures regained their original amounts of water within *ca.* 1 hr., after which no further change occurred. *X*-Ray photographs of one such mixture, taken by Dr. M. S. Webster at the Chemical Crystallography Laboratory, Oxford, showed two crystalline modifications to be present, both probably orthorhombic. The approximate unit cell dimensions are (i) *a* 7·3, *b* 6·0, *c* 38·5 Å, and (ii) *a* 11·85, *b* 5·92, *c* 25·3 Å. The two modifications were not distinguished in the density measurements which gave $\rho = 1{\cdot}394$, and calculations based on this value gave $M = 354$ and 373 for (i) and (ii) respectively. $C_{20}H_{22}O_6$ and $C_{20}H_{22}O_6,H_2O$ require M, 358·4 and 376·4 respectively. The anhydrous form showed bands at 1464, 1397, 1389, 1364, 1337, 1285 (shoulder at ~1304), 1178 (shoulder at ~1190), 1160, 1132, 1090 (shoulder at ~1101), 1075, 1064, 1045, 1030, 1015, 995, 971, 955, 918, 906, 890, 871, 861 (shoulder at ~847), 828, 813, 780, 758, 735, 725, and 686 cm.$^{-1}$, in addition to those already discussed (see Figure).

Mircestrol is readily soluble in methanol and in dioxan, sparingly soluble in ethyl acetate and in ether, and almost insoluble in benzene and in chloroform; the solubility in water is 90 mg./l. at 28°. It gives no colour with ferric chloride solution. The compound is stable under neutral conditions, solutions in methanol having undergone little change when kept for long periods in a refrigerator.

Paper Chromatography.—Compounds were run at 28°, by the descending method, on Whatman No. 2 filter paper. The following solvent systems, of the type described by Bush,[15] were used: toluene–ethyl acetate–methanol–water (9 : 1 : 5 : 5 v/v) (C system); and toluene–ethyl acetate–methanol–water (1 : 1 : 1 : 1 v/v) (G2 system). When sprayed with 2% sodium periodate solution adjusted to pH 4, chromatograms of mircestrol (R_F 0·5, G2 system), mircestrol 3-acetate, 2-bromomircestrol, isomircestrol (at 50°), and isomircestrol methyl hemiacetal (at 50°) gave yellow-green spots, fluorescent in ultraviolet light; mircestrol 3,18β(?)-diacetate gave a similar spot after preliminary treatment of the chromatogram with 5% aqueous potassium carbonate at 20°. Alternatively, spraying was carried out with 5% aqueous potassium carbonate followed, after drying, with a solution of diazotised *p*-aminophenyl 2-diethylaminoethyl sulphone; chromatograms of the above compounds, and of the dibromo-methoxy-derivative and potassium borohydride reduction product, then gave pink or reddish-brown spots. By means of the above reagents, each of these compounds could be detected in amounts of *ca.* 5 μg.

14,17, 18β-*Trihydroxy*-3-*methoxymircestra*-1,3,5,7-*tetraen*-15-*one* (*Mircestrol* 3-*Methyl Ether*).—(*a*) A solution of mircestrol (40·6 mg.) and dimethyl sulphate (0·021 ml.) in acetone (15 ml.) containing potassium carbonate (540 mg.) was refluxed in nitrogen. After 4 hr. dimethyl sulphate (0·016 ml.) was added, and refluxing was continued for a further 3 hr. The mixture was evaporated to dryness *in vacuo* at 30°, water (8 ml.) was added, and after 3 hr. in nitrogen

[15] Bush, *Biochem. J.*, 1952, **50**, 370.

the mixture was filtered, giving crystals (35·6 mg.), m. p. 205—263°. Crystallisation from dioxan gave the *methyl ether* (29·8 mg., 71%) as prisms, m. p. 271° (slight decomp.) (Found: C, 67·4; H, 6·7; OMe, 10·9. $C_{20}H_{21}O_5$·OMe requires C, 67·75; H, 6·5; OMe, 8·3%), much less soluble in methanol than is mircestrol.

(*b*) A solution of mircestrol (23·4 mg.) in methanol (6 ml.) was treated with diazomethane in ether, until a portion of the mixture gave only a faint colour with a solution of diazotised *p*-aminophenyl 2-diethylaminoethyl sulphone. Evaporation gave a solid which was dissolved in ethyl acetate, washed with N-aqueous sodium hydroxide, followed by water, dried (Na_2SO_4), and recovered. Fractional crystallisation of the residue (21 mg.) gave mircestrol 3-methyl ether (1 mg.) as plates (from ethyl acetate), m. p. 269—271°.

Reaction of Mircestrol with Excess of Diazomethane.—Mircestrol (30 mg.) in ethanol (2·5 ml.) was treated with diazomethane (*ca.* 20 mol.) in ether, and after 30 min. the solution was filtered and evaporated. The residue was dissolved in ethyl acetate, washed with N-sodium hydroxide, followed by water, dried (Na_2SO_4), and recovered as a solid (30·5 mg.). Crystallisation from toluene (6 ml.) furnished a solid *methyl ether* (4·7 mg.), m. p. 179—202°, which crystallised from propan-1-ol as needles (2·9 mg.), m. p. 203—206°. Another recrystallisation from propan-1-ol brought the m. p. to 204—205°. The infrared absorption spectrum includes bands at 3521, 2941 (shoulders at ~2865 and ~2833), 1715 (C=O), 1666 (–O–CH=C<), 1623, 1585, and 1506 $cm.^{-1}$ (last three bands due to the benzene ring). The toluene filtrate gave only mixtures.

Acetylation of Mircestrol.—A solution of mircestrol (20 mg.) in pyridine (0·15 ml.) and benzene (0·12 ml.) containing acetic anhydride (0·014 ml., 2·6 mol.) was kept at 20° for 21 hr. in nitrogen, then evaporated at 20° with a stream of nitrogen, and the residue was dried *in vacuo*. Paper chromatography of the residue in system C disclosed two products. Crystallisation from methanol gave needles (5·5 mg.), m. p. 235—242°, which crystallised from ethanol–ethyl acetate giving 3-*acetoxy*-14,17,18β-*trihydroxymircestra*-1,3,5,7-*tetraen*-15-*one* (*mircestrol* 3-*acetate*) as plates, m. p. 235—242° (slight decomp.) (Found: C, 66·2; H, 5·8. $C_{22}H_{24}O_7$ requires C, 66·0; H, 6·0%). The residual materials were fractionally crystallised in an attempt to isolate the mircestrol diacetate, but the latter decomposed. In another experiment, mircestrol (14·7 mg.) was treated with acetic anhydride (2·6 mol.) as described above, and the solution was evaporated with a stream of nitrogen. The residue was dissolved in ethyl acetate, washed with 0·2N-hydrochloric acid, water, 0·2N-sodium hydrogen carbonate, and water, dried (Na_2SO_4), and recovered *in vacuo* at 40°. Crystallisation of the residue (16·6 mg.) from methanol (0·5 ml.) gave impure mircestrol 3-acetate (2 mg.). The filtrate was evaporated and the residue warmed with the mobile phase (4 ml.) of solvent system C; filtration gave impure mircestrol (2·7 mg.). The filtrate was poured on to a partition chromatographic column (30 × 1·1 cm.) prepared from kieselguhr and solvent system C. The column was eluted with the mobile phase and the eluate collected in 3 ml. fractions; evaporation of the 8th and 9th fractions gave a sticky solid (9·9 mg.) which was extracted with 50% aqueous methanol (1 ml.). The extract was evaporated and the residue crystallised from toluene–light petroleum (b. p. 40—60°), giving *mircestrol* 3,18β(?)-*diacetate* (5·7 mg.) as rods, m. p. 184—190° (slight decomp.), unchanged by further recrystallisation from the same solvent (Found: C, 64·65; H, 5·9. $C_{24}H_{26}O_8$ requires C, 65·15; H, 5·9%).

Bromination of Mircestrol.—To a solution of mircestrol (108·4 mg.) in methanol (8 ml.), cooled in ice, was added dropwise, with shaking, in 4 min. a standardised solution of bromine in carbon tetrachloride (1·45 mol. in 3·8 ml.). The solution was poured into 0·5% aqueous sodium hydrogen carbonate (150 ml.), and the mixture was extracted with ethyl acetate (total, 180 ml.). The extracts were washed with 1% aqueous sodium hydrogen carbonate to remove coloured products, then with water, dried (Na_2SO_4), and evaporated *in vacuo* at 40°. The residual solid (145·8 mg.) was subjected to partition chromatography on kieselguhr (204 g.; 104 × 2·3 cm.) with the solvent system toluene–ethyl acetate–methanol–water (8 : 2 : 5 : 5 v/v). The solid, dissolved in a mixture of mobile phase (4 ml.) and stationary phase (17 ml.), was added to the column, which was eluted with mobile phase; after 150 ml. of eluate had flowed, it was collected in 15 ml. fractions, each fraction being evaporated to dryness and the residue chromatographed on paper in system G2. The residues from fractions 34—39 (30 mg.), consisting mainly of a product of R_F 0·7, were washed with toluene (2 + 1 ml.), and the remaining solid (24 mg.) was crystallised from methanol, giving 2-*bromo*-3,14,17,18β-*tetrahydroxymircestra*-1,3,5,7-*tetraen*-15-*one* (2-*bromomircestrol*) (I) (13·6 mg.) as needles, which slowly blackened in air above *ca.* 237° (Found: C, 55·1; H, 5·1; Br, 17·7. $C_{20}H_{21}O_6Br$ requires

C, 54·9; H, 4·8; Br, 18·25%). The combined residues from fractions 12—22 (89·4 mg.), consisting mainly of a product of R_F 0·85, were washed with toluene (3 + 2 ml.) and recrystallised from methanol, which gave almost colourless prisms (61·4 mg.). A second recrystallisation from methanol gave a *dibromo-methoxy-derivative* (44·1 mg.) as prisms, which slowly blackened in air above *ca.* 190°; this material was indicated by paper chromatography to be homogeneous (Found: C, 45·9; H, 4·2; Br, 28·6, 29·05; OMe, 8·5. $C_{20}H_{21}O_6Br_2$·OMe requires C, 46·0; H, 4·4; Br, 29·2; OMe, 5·7%). The infrared absorption spectrum includes bands at 3436, 2898, 1618, 1589, and 1488 cm.$^{-1}$ (last three bands due to a benzene ring).

Conversion of 2-Bromomircestrol (I) *into the Dibromo-methoxy-derivative.*—To a solution of 2-bromomircestrol (1·7 mg.) in methanol (0·8 ml.) at 0° was added bromine (1 mol.) in carbon tetrachloride (0·7 ml.), and the products were worked up similarly to the products of bromination of mircestrol. The isolated solid (2·5 mg.) was chromatographed on paper in solvents G2; spraying the chromatogram with diazotised *p*-aminophenyl 2-diethylaminoethyl sulphone gave a single spot, identical with that given by the pure dibromo-methoxy-derivative, which was run similarly. The relative size of the spots indicated virtually all the solid to consist of the dibromo-methoxy-derivative.

Stability of the Dibromo-methoxy-derivative towards Alkali.—A solution of the dibromo-methoxy-derivative (2·4 mg.) in 10% ethanolic potassium hydroxide was refluxed in nitrogen for 25 min. The orange solution was cooled and neutralised with dilute nitric acid, and the ethanol was evaporated *in vacuo*. Filtration then gave needles (1·8 mg.), which slowly blackened above *ca.* 180°. Paper partition chromatography of this material in the usual way, in the G2 system, indicated it to consist largely of the dibromo-methoxy-derivative.

Isomircestrol.—(*a*) Hydrochloric acid (*d* 1·18; 0·36 ml.) was added to a solution of mircestrol (39·9 mg.) in dioxan (4·5 ml.) and water (4·5 ml.), and the mixture was kept at 65° in nitrogen for 1·5 hr. The colourless solution was cooled and neutralised with solid potassium hydrogen carbonate, and most of the dioxan was distilled off *in vacuo* at 20°. The resulting suspension was diluted with 1% aqueous sodium hydrogen carbonate and extracted with ethyl acetate; the extracts were washed with water, dried (Na_2SO_4), and evaporated *in vacuo* at 40°. The residue (30·4 mg.), m. p. 215—220°, on two recrystallisations from ethanol–ethyl acetate, gave *isomircestrol* (20 mg.) as slender rods, m. p. 220—222° (slight decomp.). Analyses of specimens crystallised from the latter solvent, and also from methanol, gave inconsistent results because, as shown by desiccation experiments, these specimens contained varying amounts of a hydrate derived from a very hygroscopic form of isomircestrol. Crystallisation from 20% aqueous ethanol appeared to give mainly a *monohydrate*, as needles, m. p. 220—223° (slight decomp.) (Found: C, 64·4; H, 6·2. $C_{20}H_{22}O_6,H_2O$ requires C, 63·8; H, 6·4%). Zeisel analysis gave an apparent 6·65% of methoxyl. A specimen, shielded from light, was dried at 100°/2 mm. to constant weight (30 min.); the desiccated material took up water on admission of air, the original weight being regained within 2 hr., after which no further change took place. A repetition of this experiment on the same specimen gave the same result (Found: loss of wt., 4·4. Calc. for $C_{20}H_{22}O_6,H_2O$: H_2O, 4·8%). The infrared absorption spectrum of a partially hydrated specimen included bands at 3534, 3401, 2959 (shoulders at ~2865 and ~2825), 1678, 1623 (shoulder at ~1597), and 1505 cm.$^{-1}$ (last 3 bands due to a benzene ring).

(*b*) To a solution of mircestrol (5·4 mg.) in dry dioxan (1·5 ml.) was added a 7·5% w/v solution of hydrogen chloride in dry dioxan (0·75 ml.). After 5 hr. the mixture was poured into 1% aqueous sodium hydrogen carbonate (30 ml.) and, after 30 min., the products were extracted with ethyl acetate. This gave a solid (5·1 mg.) shown by paper chromatography in the G2 system to consist almost entirely of isomircestrol, with no mircestrol. Crystallisation from ethanol–toluene gave isomircestrol (1·2 mg.), m. p. 217—222°, undepressed on admixture with a specimen prepared as in (*a*).

(*c*) To a solution of isomircestrol methyl hemiacetal (see below) (3·4 mg.) in dioxan (1 ml.) and water (3 ml.) hydrochloric acid (*d* 1·18; 0·1 ml.) was added, and the mixture was kept at 65° in nitrogen for 1·5 hr., then poured into 1% aqueous sodium hydrogen carbonate; the products were extracted into ethyl acetate, washed with water, dried (Na_2SO_4), and recovered as a solid (3·4 mg.), m. p. 205—215°. Crystallisation of the solid from ethyl methyl ketone gave isomircestrol, m. p. 223°, undepressed by admixture with a specimen prepared as in (*a*).

Isomerisation of Mircestrol 3-Methyl Ether.—To a solution of this ether (29·8 mg.) in dioxan (9 ml.) and water (1·5 ml.) hydrochloric acid (*d* 1·18; 0·5 ml.) was added, and the mixture was kept at 75° in nitrogen for 2¼ hr., cooled, diluted with water (4 ml.), neutralised with solid

potassium hydrogen carbonate, and evaporated *in vacuo* at 40° to 3 ml. Filtration gave a solid (28·8 mg.), m. p. 232—237°, which was recrystallised from 2-methoxyethanol, giving an *isomircestrol monomethyl ether* (22·2 mg.) as plates, m. p. 232—234° (slight decomp.), sparingly soluble in methanol and insoluble in N-aqueous sodium hydroxide (Found: C, 67·5; H, 6·6. $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·5%), $\nu_{max.}$ 3448, 3220, 2955 (shoulder at ~2915), 1674, 1616, 1581, and 1504 cm.$^{-1}$.

Isomircestrol Methyl Hemiacetal.—(*a*) To a solution of mircestrol (34·8 mg.) in methanol (10 ml.) was added hydrochloric acid (*d* 1·18; 0·2 ml.), and the mixture was kept in nitrogen for 4 hr. The solution was poured into 0·5% aqueous sodium hydrogen carbonate, and the products were isolated in ethyl acetate. This gave a solid (38·3 mg.), m. p. 175—182°, which crystallised from ethyl acetate giving *isomircestrol methyl hemiacetal* (29·8 mg.) as rods, m. p. 178—181° (slight decomp.). This material (shielded from light, since it was light-sensitive at elevated temperatures) was dried at 110°/0·5 mm. to constant weight (1·5 hr.) (Found: loss of wt., 2·95. $C_{21}H_{24}O_6,H_2O$ requires H_2O, 4·6%). On exposure to air, a regain of weight of 2·2% occurred during 5 days, after which the weight continued to increase, very slowly. The specimen therefore appeared to consist partly of a hydrate derived from a weakly hygroscopic form of isomircestrol methyl hemiacetal. This specimen was accordingly desiccated as before, and analysed shortly afterwards (Found: C, 67·1; H, 6·85; OMe, 11·7. $C_{20}H_{21}O_5$·OMe requires C, 67·75; H, 6·5; OMe, 8·35%). An attempt to prepare a definite hydrate by crystallisation of the acetal from 10% aqueous methanol was unsuccessful, since the compound again appeared to crystallise only partly as a hydrate. The infrared absorption spectrum of a partially hydrated specimen included bands at 3448, 2947 (shoulder at ~2837), 1678, 1623 (shoulder at ~1597) and 1505 cm.$^{-1}$. The compound is soluble in dilute aqueous sodium hydroxide.

(*b*) To a solution of isomircestrol (10 mg.) in methanol (3 ml.) was added hydrochloric acid (*d* 1·18; 0·06 ml.). After 8 hr., the solution was poured into 0·5% aqueous sodium hydrogen carbonate (70 ml.), and the products were isolated by extraction with ethyl acetate. This gave a solid (7 mg.), m. p. 144—148°, which crystallised from ethyl acetate, giving the hemiacetal (3 mg.), m. p. 176—179°, undepressed on admixture with a specimen prepared as in (*a*).

Stability of Mircestrol towards Alkali in Nitrogen.—(*a*) A solution of mircestrol (1·5 mg.) in aqueous-ethanolic potassium hydroxide (25% KOH w/v; 1 ml.) was kept at 20° for 15 min., and then at 55° for 2 min., in nitrogen. The green solution was cooled in nitrogen, neutralised, and extracted with ethyl acetate. The extract gave a solid (1·7 mg.) which chromatography on paper with the G2 system showed to be largely unchanged mircestrol and to contain no other phenol.

(*b*) Mircestrol (4·1 mg.) was treated with alkali and the products were worked up as in (*a*), except that the alkaline solution was kept at 70° for 20 min. The isolated solid (4·1 mg.) was chromatographed on paper in the G2 system, which showed it to consist mainly of a phenol giving a red-brown spot, R_F 0·9, on treatment with a diazotised solution of *p*-aminophenyl 2-diethylaminoethyl sulphone; no unchanged mircestrol was detected. An attempt to isolate the phenol, R_F 0·9, by crystallisation was unsuccessful.

Reduction of Mircestrol by Potassium Borohydride.—To a solution of mircestrol (23·4 mg.) in methanol was added potassium borohydride (183 mg.) in methanol (7 ml.), and the mixture was kept in nitrogen. After 70 min., potassium borohydride (138 mg.) was added, and the mixture was kept, with occasional shaking, in nitrogen for a further 50 min. (A large excess of reducing agent was used, to ensure completion of the reaction.) Methanol was then added to dissolve the residual solid; to the solution was added acetone (3 ml.) and water (4 ml.), and the mixture was left at 0° overnight. The organic solvents were evaporated *in vacuo* at 20°, and the residual solution was neutralised at 0° with 2N-hydrochloric acid. The precipitated solid (21·9 mg.) was filtered off, and chromatographed in the G2 system; this indicated only one phenolic product, R_F 0·7, to be present, and no unchanged mircestrol was detected. To remove a little inorganic material, the solid was shaken with ethyl acetate, and the mixture filtered, washed with water, dried (Na_2SO_4), and evaporated to dryness. The solid residue (15·2 mg.) was recrystallised twice from ethyl acetate, which gave a reduction *product* (8 mg.) as prisms, m. p. 248—252° (slight decomp.), unchanged by further recrystallisation from the same solvent (Found: C, 69·1; H, 6·4. $C_{20}H_{22}O_5$ requires C, 69·35; H, 6·4%), $\nu_{max.}$ at 3522, 3356, 2950, 1622, 1595, and 1504 cm.$^{-1}$ (last 3 bands due to a benzene ring).

Reducing Properties of Mircestrol and Derivatives.—Fehling's test was carried out, in nitrogen,

using Fehling's procedure as adapted by Nelson and Somogyi [16] for the colorimetric estimation of glucose. Tests with hot, alkaline solutions of triphenyltetrazolium bromide were carried out, also in nitrogen, by Feigl's [17] method. In addition to the results described in the discussion, it was found that the methyl ether, m. p. 203—206°, formed by reaction of mircestrol with excess of diazomethane, does not reduce Fehling's solution.

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