97. *The Synthesis of Compounds related to the Sterols, Bile Acids, and Oestrus-producing Hormones. Part VI. Experimental Evidence of the Complete Structure of Oestrin, Equilin, and Equilenin.*

By A. Cohen, J. W. Cook, and C. L. Hewett.

In Part III (Cohen, Cook, Hewett, and Girard, J., 1934, 653) proof was adduced that the ring system of oestrin * and equilenin is that of cholesterol, with the hydroxyl group in the same position, independent evidence of the latter feature being also obtained by Haworth and Sheldrick (*ibid.*, p. 864). The present communication deals with the location of the keto-group and the quaternary methyl group of the hormones, and in our view the experiments which we now record not only establish a complete correspondence in molecular structure of the three oestrogenic hormones, oestrone, equilin, and equilenin, but also demonstrate conclusively that the keto-group is at position 17 and the quaternary methyl group at position 13 (sterol system of numbering). Consequently, the only feature of the structure (as distinct from configuration) of these hormones which still remains undetermined is the position of the double bond in equilin (Girard, Sandulesco, Fridenson, Gaudefroy, and Rutgers, *Compt. rend.*, 1932, **194**, 1020). We have not attempted to secure evidence of this, as Dr. Girard has informed us that Professor L. Ruzicka is collaborating with him on this question.

Our results lend strong support to the view that the hormones represent successive stages of dehydrogenation of a sterol molecule, after oxidative removal of its side chain, from which it may be inferred that the animal body contains some factor, possibly an enzyme, capable of inducing dehydrogenation of the sterol ring system. In suitable circumstances such a factor may play an important part in the conversion of one of these

* This term is used to include both oestrone and oestriol, of which the inter-relationship has been established.

natural products into a cancer-producing compound (compare Cook and Haslewood, J., 1934, 428).

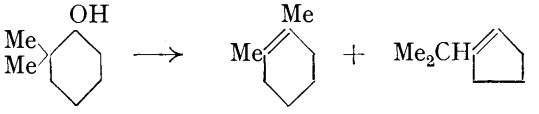
With the object of defining the position of the keto-group in oestrone, we submitted its methyl ether to the action of methylmagnesium iodide, then dehydrated the resultant carbinol, afterwards hydrogenated the double bond thus produced, and finally dehydrogenated the ring system by heating with selenium. We expected in this way to obtain a methyl derivative of 7-methoxy-1 : 2-*cyclo*pentenophenanthrene, and clearly the point of attachment of the methyl group would be that occupied by the keto-group of the original hormone.

Now, it was known from previous work (Marrian and Haslewood, *Chem. and Ind.*, 1932, **51**, 277T; Butenandt, Weidlich, and Thompson, *Ber.*, 1933, **66**, 601) that the two alcoholic hydroxyl groups of oestriol, and therefore the keto-group of oestrone, are in the five-membered ring. Consequently, the product of the above series of reactions should have been the 1′-, 2′-, or 3′-methyl derivative of 7-methoxy-1 : 2-*cyclo*pentenophenanthrene. Of these, the 3′-methyl structure was to be preferred, as this would lead to the same position in the molecule for the keto-group of oestrone as that occupied by the side chain of the sterols and bile acids. However, the product from oestrone, which was also obtained in equally good yield from equilin and equilenin, was different from synthetic specimens of all three of these methyl derivatives of 7-methoxy-1 : 2-*cyclo*pentenophenanthrene.

For the synthesis of the 1′- and the 2′-methyl compound we employed an analogous method to that used by Ruzicka, Ehmann, Goldberg, and Hösli (*Helv. Chim. Acta*, 1933, **16**, 833) for the synthesis of 1′- and 2′-methyl-1 : 2-*cyclo*pentenophenanthrene. For the 3′-methyl compound we employed a method analogous to Kon's (Harper, Kon, and Ruzicka, J., 1934, 124) adaptation of the synthetic method used by Cook and Hewett (J., 1933, 1098) for 1 : 2-*cyclo*pentenophenanthrene. The following table, giving the melting points of the synthetic derivatives of 7-methoxy-1 : 2-*cyclo*pentenophenanthrene and their *s*.-trinitrobenzene complexes, together with those of the analogous compounds prepared from the hormones, shows that these constants are sufficient to characterise the compounds, and to establish their individuality.

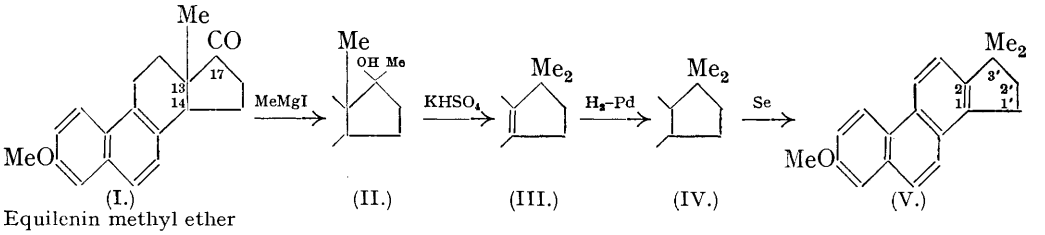
	Synthetic derivatives of 7-methoxy-1 : 2-*cyclo*pentenophenanthrene.				Product from
	Parent compound.	1′-Me.	2′-Me.	3′-Me.	hormones.
M. p. of methoxy-compound...	136° (corr.)	98°	137°	148°	166°
M. p. of *s*.-trinitrobenzene complex	161 (corr.)	129	133	137	174

The divergence between the product from the hormones and the three synthetic methyl compounds was apparent from a consideration of the most probable structures of the hormones, for in the carbinols arising from the methyl ethers of the hormones by treatment with methylmagnesium iodide the carbinol group is adjacent to a quaternary carbon atom, provided that the quaternary methyl group is at position 13 and the original keto-group at position 17. But it is well known that dehydration of carbinols of this type is attended by group migration, a somewhat comparable example being found in the dehydration of 2 : 2-dimethyl*cyclo*hexanol, which gives 75% of 1 : 2-dimethyl-Δ^1-*cyclo*hexene and 25% of 1-*iso*propyl-Δ^1-*cyclo*pentene (Meerwein, *Annalen*, 1914, **405**, 129).



By analogy, dehydration of the carbinols obtained from the hormones involves simultaneous migration of the quaternary methyl group to the adjacent position in the five-membered ring, so that the final product is 7-*methoxy*-3′ : 3′-*dimethyl*-1 : 2-cyclo*pentenophenanthrene* (V), the elementary composition of which is indistinguishable from that of a monomethyl compound. In the series of transformations with equilenin

the pure product was isolated at each stage, and the changes may be formulated as follows:

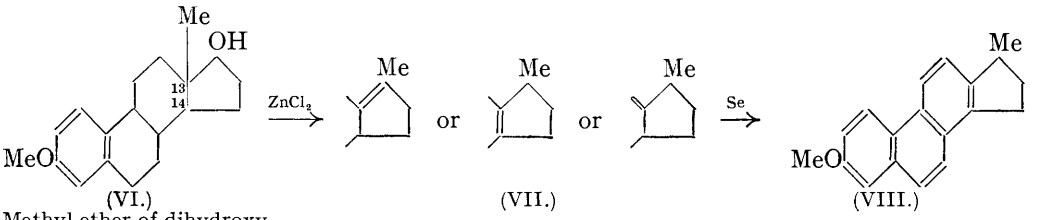


(I.) (II.) (III.) (IV.) (V.)

Equilenin methyl ether

Hydrogenation of the double bond prior to selenium dehydrogenation was effected in order to avoid possible complications due to unsaturation in the five-membered ring. When the reactions with equilin were carried out, the true nature of the changes had been appreciated and it was realised that this previous hydrogenation was superfluous, so the dehydration product of the carbinol was treated directly with selenium. It is significant that the pure, sublimed, and recrystallised product was isolated in 55% yield after heating with selenium at 295—305° for only 7 hours.

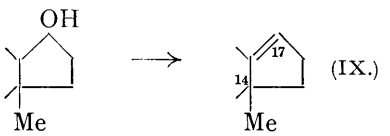
Conclusive proof of the correctness of the above interpretation of the hormone transformations was secured by an experiment with the dihydroxyoestratriene which arises by reduction of oestrone (Girard, Sandulesco, and Fridenson, *Compt. rend. Soc. Biol.*, **1933**, **112**, 964). The methyl ether of this secondary carbinol would clearly be expected to behave, on dehydration, like the tertiary carbinols already discussed, and this was, in fact, the case. Selenium dehydrogenation of its dehydration product led to *7-methoxy-3'-methyl-1 : 2-cyclopentenophenanthrene* (VIII), identical with the synthetic sample of this compound:



(VI.) (VII.) (VIII.)

Methyl ether of dihydroxy-oestratriene

The methyl group which appears at position 3' in the final product is the quaternary methyl group of the original oestrone molecule, and we regard these transformations as supplying indisputable proof of position **13** for this methyl group, for if it were at **14** (the only alternative for equilenin) there would be a free hydrogen atom at position **13**, and *normal* dehydration of the carbinol would ensue:



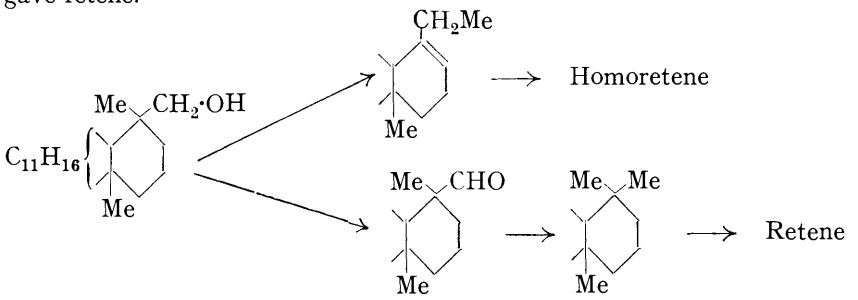
(IX.)

The possibility of migration of methyl from C_{14} to C_{17} during dehydrogenation of a structure such as (IX) is discounted by the experiments with the tertiary carbinols from oestrone and equilenin, when care was taken to hydrogenate the double bond before dehydrogenation of the ring system.

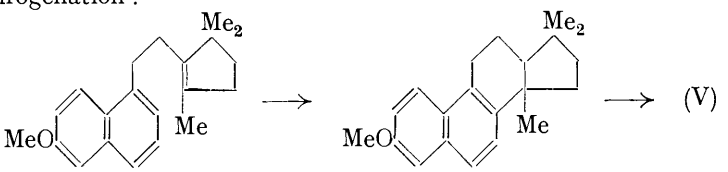
We may recall that it was shown in Part III (*loc. cit.*) that, when the keto-group of oestrone or equilenin was reduced (Kishner–Wolff) to a methylene group and the methyl ether of the product then dehydrogenated, the quaternary methyl group was *eliminated*, leading to 7-methoxy-1 : 2-*cyclo*pentenophenanthrene.

These changes, which thus involve methyl migration by one route to the aromatic

structure, and methyl elimination by another route, find an exact counterpart in some experiments made by Ruzicka and his collaborators (*Helv. Chim. Acta*, 1922, **5**, 581; 1932, **15**, 1300; 1933, **16**, 169) with abietinol, the Bouveault reduction product of the methyl ester of abietic acid. These authors showed that the methyl group migration which accompanied the dehydration of abietinol could be avoided by oxidation to abietinal, followed by Kishner–Wolff reduction of this aldehyde. The hydrocarbon obtained by the first route gave homoretene on dehydrogenation, whereas that obtained by the second method gave retene.



The structure (V) which we assign to the dimethyl derivative of 7-methoxy-1 : 2-*cyclo*pentenophenanthrene receives confirmation by its synthesis by condensation of β-6-methoxy-1-naphthylethylmagnesium bromide with 2 : 2 : 5-trimethyl*cyclo*pentanone, followed by dehydration of the crude carbinol, cyclisation of the product, and then selenium dehydrogenation :



We are indebted to Dr. John Iball for a preliminary *X*-ray crystallographic examination of 7-methoxy-3′ : 3′-dimethyl-1 : 2-*cyclo*pentenophenanthrene from oestrone. *X*-Ray rotation and oscillation photographs gave the following dimensions of the monoclinic cell :

$$a = 8{\cdot}75 \text{ Å.}; \quad b = 6{\cdot}21 \text{ Å.}; \quad c = 28{\cdot}0 \text{ Å.}; \quad \beta = 90°.$$

The density of the crystal, measured by the flotation method, was 1·188 at 20°. Assuming 4 molecules per unit cell, these values give 274 ± 1% for the molecular weight, which confirms the theoretical value of 276 for $C_{20}H_{20}O$. The lattice seems to be of the usual layer type with an almost flat molecule, the above cell dimensions being approximately in the directions of the width, thickness, and length, respectively, of the molecules.

Discussion.

The results which we have obtained have a bearing on several problems connected with the sterols and bile acids. For example, the two most important bile acids (cholic and deoxycholic acids) have a hydroxyl group at C_{12}, *i.e.*, the position in ring III adjacent to the quaternary methyl group at C_{13}, and an investigation of the dehydration of certain derivatives of these bile acids is in progress in this laboratory, as it seems likely that methyl migration from C_{13} to C_{12} will be encountered. Moreover, it is now possible to present a rational interpretation of the formation of 3′-methyl-1 : 2-*cyclo*pentenophenanthrene by dehydrogenation of sterols, bile acids, and cardiac aglucones. Clearly the methyl group migration from C_{13} to C_{17} is concomitant with, and inseparable from, the elimination of the side chain, and is in no way dependent on the dehydrogenation. The side chain may be regarded as being expelled from the molecule as an anion; the whole process then becomes a special case of the transformations which include the pinacolin and Wagner–Meerwein rearrangements (compare Robinson, *Mem. Manchester Phil. Soc.*, 1920, **64**,

No. 4, 1). Support for this view is afforded by the observation of Diels, Gädke, and Körding (*Annalen*, 1927, **459**, 1), which we have confirmed, that 3′-methyl-1 : 2-*cyclo*pentenophenanthrene ("$C_{18}H_{16}$") is obtained not only by dehydrogenation of cholesterol, or better cholesteryl chloride, but also from the hydrocarbon "$C_{19}H_{18}$" first obtained by Mauthner and Suida (*Monatsh.*, 1896, **17**, 41) by pyrolysis of cholesteryl chloride. In this last process the intact side chain is eliminated, as was clearly shown by the subsequent re-investigation by Fischer and Treibs (*Annalen*, 1926, **446**, 257). We are of the opinion that there are very few examples of group migration which may be ascribed unequivocally to the action of dehydrogenating catalysts, for the well-known migrations which have been observed with various *gem*-dimethyl derivatives of *cyclo*hexane and tetralin have usually occurred under the influence of bromine or nitric acid (compare Baeyer and Villiger, *Ber.*, 1899, **32**, 2429; Crossley, J., 1904, **85**, 264; Crossley and Renouf, J., 1909, **95**, 930; Inhoffen, *Annalen*, 1932, **497**, 134). This undoubtedly involves substitution of, for example, bromine for hydrogen, as an intermediate phase. Elimination of hydrogen bromide from a compound in which bromine occupies an adjacent position to a *gem*-dimethyl group would be just as certainly attended by methyl migration as the dehydration of carbinols of analogous structure. The important point, which we believe is not generally appreciated, is that the migration is not determined *ipso facto* by the dehydrogenation.

The position which we assign on the basis of our present experiments to the quaternary methyl group of the oestrogenic hormones is that which is now fairly generally accepted for one of the quaternary methyl groups in the sterol-bile acid molecule, but as the direct conversion of a sterol into one of the hormones has not yet been achieved we naturally do not claim that our evidence supplies confirmation of this position for the sterol molecule. However, the theoretical considerations underlying the evidence for placing the methyl group at C_{13} in the bile acid molecule (Wieland and Dane, *Z. physiol. Chem.*, 1933, **216**, 91; compare Wieland and Schlichting, *ibid.*, 1924, **134**, 276) have been verified experimentally by the recent work of Cook and Linstead (J., 1934, 946) and we regard the structures of cholesterol and the bile acids as being now completely established in every detail, for if once position C_{13} rather than C_{14} is accepted for one quaternary methyl group the other must be at C_{10} (a position already strongly supported by indirect evidence) to account for the isolation of *n*-butane-$\alpha\gamma\gamma$-tricarboxylic acid from the oxidation products of pyrodeoxybilianic acid (Wieland and Vocke, *Z. physiol. Chem.*, 1928, **177**, 68).

Methods of synthesis of equilenin and allied substances have been explored in this laboratory for some time past, and attention will be concentrated on these investigations now that the constitution of this hormone has been established.

Experimental.

With five exceptions (denoted by asterisks) the analyses were microanalyses by Dr. A. Schoeller.

Experiments with Oestrone.

Oestrone Methyl Ether.—This was obtained from the hormone (2 g.) by heating on the water-bath for 2 hours with methyl *p*-toluenesulphonate (2·6 g.) and 10% aqueous potassium hydroxide (8 c.c.), a second portion (10 c.c.) being added after an hour. The methyl ether was collected, washed, and recrystallised from aqueous alcohol (charcoal). The product (1·7 g.) had m. p. 167·5—169° (compare Butenandt, Störmer, and Westphal, *Z. physiol. Chem.*, 1932, **208**, 167).

Methylcarbinol from Oestrone Methyl Ether.—The finely powdered methoxy-compound (1·5 g.) was gradually added to an ice-cold Grignard solution prepared from methyl iodide (1 c.c.), magnesium turnings (0·4 g.), and anhydrous ether (10 c.c.). After warming to room temperature, the resultant solution was boiled for 2 hours, decomposed with ice and ammonium chloride, and the ether removed by distillation. The crude carbinol was collected (m. p. 95—100°) and used without purification.

Dehydration. The product (1·5 g.) of the Grignard reaction was heated at 160—165° for 1½ hours with potassium hydrogen sulphate (3 g.). The resinous product was isolated with ether, and distilled at 0·2 mm. from a small flask immersed in an oil-bath at 190—200°. The viscous distillate was obtained crystalline (0·6 g.) from alcohol, and after recrystallisation from alcohol had m. p. 58—60° to a cloudy liquid, clear at 80°. This indefinite m. p. suggested a

mixture of isomerides, for the analytical figures were in good agreement with a structure of type (III) (Found: C, 84·9; H, 9·0. $C_{20}H_{26}O$ requires C, 85·0; H, 9·3%). A higher fraction was obtained from the above distillation by removing the last traces of material in an air-bath. After two recrystallisations from alcohol this (0·1 g.) gave pure oestrone methyl ether (in experiments with the other two hormones the technique was improved and the carbinols obtained from the Grignard reactions were successfully freed from unchanged ketone).

Catalytic Hydrogenation (this operation and also the hydrogenation of the analogous compound in the equilenin series were performed by Mr. G. A. D. Haslewood).—A solution of the aforesaid dehydration product (0·5 g.) in moist ether was shaken for 7 hours with hydrogen and a palladium-black catalyst of proved activity. The resulting colourless gum would not crystallise.

Dehydrogenation. The foregoing gum was heated with selenium (1 g.) at 300—320° for 21½ hours. The crystalline product was extracted with benzene, the solvent removed from the filtered extract, and the residue heated with a little sodium at 180° for a few minutes. The substance was freed from sodium by a second extraction with benzene, and then distilled at 0·15 mm. (air-bath at 140—160°). The distillate was recrystallised from benzene–alcohol (yield, 0·32 g.; m. p. 163—164°) and then twice from alcohol. 7-*Methoxy*-3′ : 3′-*dimethyl*-1 : 2-cyclo*pentenophenanthrene* (V) formed colourless plates (from alcohol) or needles (from benzene), m. p. 166—167°, and had no detectable optical activity (1·4% solution in dioxan) (Found: C, 86·8; H, 7·2; *M*, Rast method, 254, 261. $C_{20}H_{20}O$ requires C, 86·9; H, 7·3%; *M*, 276). This compound, like 7-methoxy-1 : 2-*cyclo*pentenophenanthrene (Part III, *loc. cit.*) and its three synthetic monomethyl derivatives (below), gave a transient carmine colour in concentrated sulphuric acid. The subsequent colour changes were similar with all five compounds, but they differed from one another in the persistence of the intermediate yellow phase and in the final colour (for example, blue-green, olive-green, or murky, but without definite colour).

The s.-*trinitrobenzene* complex of 7-methoxy-3′ : 3′-dimethyl-1 : 2-*cyclo*pentenophenanthrene crystallised from alcohol in golden-orange needles, m. p. 174—175°, and gave a large depression of the m. p. (161°) of the analogous derivative of 7-methoxy-1 : 2-*cyclo*pentenophenanthrene (Found: C, 63·5; H, 4·7. $C_{20}H_{20}O,C_6H_3O_6N_3$ requires C, 63·8; H, 4·7%).

Methyl Ether of Dihydroxyoestratriene (VI).—Sodium (1 g.) was gradually added to a solution of oestrone (1 g.) in boiling ethyl alcohol (50 c.c.). The product was recrystallised from aqueous alcohol and had m. p. 165—168° (lit. 174°) (0·7 g.). This dihydroxyoestratriene, without further purification, was methylated with methyl *p*-toluenesulphonate and 10% potassium hydroxide solution, as described for oestrone. The methyl ether could probably have been obtained in the pure crystalline state, but it was extremely soluble in all media and it was considered expedient to conserve the material for the subsequent operations.

Dehydration and dehydrogenation. The crude methoxy-carbinol (VI) (0·7 g.) was heated at 170—180° for ½ hour with powdered anhydrous zinc chloride (1·5 g.) The product, isolated by means of ether, was distilled at 0·05 mm. from an air-bath slowly heated from 115° to 140°. A smaller fraction distilled at 140—160°, and was largely crystalline. After two recrystallisations from alcohol this had m. p. 141—146°, the mixed m. p. with 7-methoxy-3′-methyl-1 : 2-*cyclo*pentenophenanthrene (m. p. 148°) being 143—147°. Thus, partial dehydrogenation had attended the dehydration.

The main distillate (0·5 g.) formed a colourless viscous liquid, and was heated with selenium, (1 g.) at 295—305° for 8½ hours. The crystalline product was extracted with benzene, heated to 170° with a little sodium, and then crystallised from alcohol. The crystals (0·35 g.) were sublimed at 150—160°/0·1 mm., and the sublimate recrystallised from alcohol, yielding 0·2 g. of pure 7-*methoxy*-3′-*methyl*-1 : 2-cyclo*pentenophenanthrene* (VIII), m. p. 146·5—147·5°. This was crystallised from alcohol with an equal weight of *s*.-trinitrobenzene. The orange needles, after recrystallisation from alcohol, had m. p. 137—138° and did not depress the m. p. of a synthetic specimen of the s.-*trinitrobenzene* complex of 7-methoxy-3′-methyl-1 : 2-*cyclo*pentenophenanthrene (below) (Found: C, 63·0; H, 4·6. $C_{19}H_{18}O,C_6H_3O_6N_3$ requires C, 63·1; H, 4·5%). The trinitrobenzene was removed from this complex by reduction with stannous chloride in boiling aqueous alcohol. The resulting 7-methoxy-3′-methyl compound (VIII) crystallised from alcohol in colourless leaflets, m. p. 147·5—148·5°, not depressed by admixture with the synthetic specimen (Found: C, 87·1; H, 7·0. $C_{19}H_{18}O$ requires C, 87·0; H, 6·9%).

Experiments with Equilenin.

Methylcarbinol from Equilenin Methyl Ether (I).—The finely powdered methyl ether (Part III, *loc. cit.*) (0·75 g.) was gradually added to an ice-cold Grignard solution prepared from methyl

iodide (1 c.c.), magnesium turnings (0·4 g.), and anhydrous ether (10 c.c.). The solution was boiled for 2½ hours, treated with ice and ammonium chloride, the ethereal solution dried (sodium sulphate), and the ether removed on the water-bath. The residual *carbinol* (II) was recrystallised from *cyclo*hexane, forming tufts of colourless silky needles (0·65 g.), m. p. 133—133·5° (Found : C, 81·3; H, 8·1. $C_{20}H_{24}O_2$ requires C, 81·0; H, 8·2%). The material isolated from the liquors was triturated with a little ether, and the undissolved residue crystallised from alcohol, yielding 30 mg. of unchanged equilenin methyl ether.

Dehydration of the methylcarbinol. This was effected by heating the pure carbinol (0·6 g.) with freshly fused potassium hydrogen sulphate (1·2 g.) at 160—170° for an hour. The *product* (III) crystallised from alcohol (charcoal) in colourless leaflets (0·4 g.), m. p. 131—132° (Found : C, 86·25; H, 7·85. $C_{20}H_{22}O$ requires C, 86·3; H, 8·0%).

Hydrogenation. This was effected in presence of palladium-black and moist ether. After two crystallisations from alcohol the saturated *methoxy*-compound (IV) formed small colourless plates, m. p. 131—132° (Found : C, 85·7; H, 8·55. $C_{20}H_{24}O$ requires C, 85·7; H, 8·6%). There was a large depression of m. p. when this substance was mixed with the original unsaturated compound.

Dehydrogenation. The foregoing hydrogenation product (0·14 g.) was heated with selenium (0·25 g.) at 300—310° for 17 hours. The product was isolated in the usual way, sublimed in a high vacuum, and recrystallised from alcohol, giving 53 mg. of pure 7-methoxy-3′ : 3′-dimethyl-1 : 2-*cyclo*pentenophenanthrene (V), m. p. 165—165·5°. The *s.*-trinitrobenzene complex had m. p. 174—175° (Found : C, 63·5; H, 4·7. Calc. : C, 63·8; H, 4·7%). Neither of these m. p.'s was depressed by admixture with the analogous compounds from oestrone.

Experiments with Equilin.

Equilin Methyl Ether.—Methylation of equilin (1 g.) was effected with methyl *p*-toluenesulphonate and 10% potassium hydroxide solution just as described for oestrone. The *methoxy*-compound (1 g.) crystallised from alcohol (charcoal) in colourless needles, m. p. 160·5—161·5° (Found : C, 80·7; H, 7·8. $C_{19}H_{22}O_2$ requires C, 80·8; H, 7·9%).

Methylcarbinol from Equilin Methyl Ether.—The Grignard condensation with the methoxyketone (0·9 g.) was effected as in the equilenin series. The *carbinol* crystallised from benzene–light petroleum in soft colourless needles (0·7 g.), m. p. 133—134° (Found : C, 80·9; H, 8·7. $C_{20}H_{26}O_2$ requires C, 80·5; H, 8·8%). Dehydration of the carbinol (0·6 g.) was effected by heating for an hour at 160—170° with potassium hydrogen sulphate (1·2 g.). The product was isolated with ether, distilled in a high vacuum (air-bath at 140—150°), and the distillate crystallised from alcohol. The *product* (0·26 g.) formed colourless needles, m. p. 105—110°, after sintering, this figure being raised to 112—117° by recrystallisation from alcohol (Found : C, 86·2, 86·1; H, 7·7, 7·8. $C_{20}H_{24}O$ requires C, 85·7; H, 8·6%). The heterogeneous nature of this substance was undoubtedly due to the presence of dehydrogenation product, as in the case of the analogous substance from dihydroxyoestratriene. This view was confirmed by the rise in the carbon figure and the fall in the hydrogen figure shown by a fraction, m. p. 132—139°, obtained by two further recrystallisations from alcohol (Found : C, 86·7; H, 7·3%).

Dehydrogenation. The once-recrystallised dehydration product, m. p. 105—110° (0·1 g.), was heated with selenium (0·2 g.) at 295—305° for 7 hours. Pure 7-methoxy-3′ : 3′-dimethyl-1 : 2-*cyclo*pentenophenanthrene (V) (55 mg.), m. p. 164—165°, was isolated in the usual way. The *s.*-trinitrobenzene complex had m. p. 174—175° (Found : C, 63·8; H, 4·6. Calc. : C, 63·8; H, 4·7%). The identity of these compounds with the analogous compounds from oestrone was confirmed by mixed m. p. determinations.

Synthesis of 1′- *and* 2′-*Methyl Derivatives of* 7-*Methoxy*-1 : 2-cyclo*pentenophenanthrene.*

Two stages in the synthesis of β-6-methoxy-1-naphthylethyl alcohol (Part III, *loc. cit.*) were improved when the preparation was carried out on a larger scale. (a) When reduction of 1-nitro-6-methoxynaphthalene was effected with aluminium amalgam and aqueous alcohol, the amine was obtained in a satisfactory state of purity without distillation : Boiling water (40 c.c.) was added to a mixture of the nitronerolin (50 g.), amalgamated aluminium strips (55 g.), and absolute alcohol (500 c.c.). After the vigorous reaction had subsided, the whole was heated on the water-bath for 1¼ hours, three further quantities of 40 c.c. of water being added during the first ½ hour. The boiling solution was filtered from the alumina sludge, which was well washed with boiling alcohol. The alcohol was removed from the filtrate under reduced pressure, and the residual oil treated with 2*N*-sulphuric acid (115 c.c.). The resulting

sulphate was collected and recrystallised from water slightly acidified with sulphuric acid. The sulphate was then sufficiently pure for conversion into iodonerolin.

(b) The yield of methoxynaphthylethyl alcohol was improved by adding a molecular proportion of ethyl bromide during the preparation of the Grignard compound of 5-iodonerolin (compare Grignard, *Compt. rend.*, 1934, **198**, 625, 2217). A mixture of iodonerolin (50 g.), ethyl bromide (13 c.c.), and anhydrous ether (350 c.c.) was added, during 1½ hours, to magnesium turnings (8·5 g.), activated with iodine. Boiling was continued for a further 2 hours, and the solution, cooled in a freezing mixture, was slowly treated with ethylene oxide (25 g.) diluted with ether (50 c.c.). After ½ hour, the whole was allowed to warm to room temperature, kept over-night, and the ether then slowly distilled on the water-bath. The product was isolated as previously described. In this way, 85 g. of β-6-methoxy-1-naphthylethyl alcohol were obtained from 170 g. of 5-iodonerolin (yield, 70%).

β-6-*Methoxy*-1-*naphthylethyl Bromide*.—Phosphorus tribromide (4·7 g.) was added to a solution of the alcohol (10 g.) in carbon tetrachloride (10 c.c.), previously heated to 60°. After the brisk evolution of hydrogen bromide had ceased, the mixture was kept at 60° for ¼ hour, cooled, and well extracted with water and with sodium carbonate solution. The oil was distilled in a vacuum, giving the *bromide* (7·1 g.), b. p. 155°/0·4 mm., as a crystalline distillate, which was recrystallised from alcohol, forming colourless needles, m. p. 57—58° (Found: C, 58·8; H, 5·4. $C_{13}H_{13}OBr$ requires C, 58·9; H, 5·0%). The residue from the distillation was hydrolysed with alcoholic potash, and the product again treated with phosphorus tribromide, giving a further 1·9 g. of the above bromide.

Ethyl 2-(β-6′-*Methoxy*-1′-*naphthylethyl*)-5-*methyl*cyclo*pentanone*-2-*carboxylate*.—The potassio-compound prepared by 3—4 hours' boiling of ethyl 5-methyl*cyclo*pentanone-2-carboxylate (Cornubert and Borrel, *Bull. Soc. chim.*, 1930, **47**, 301) (9 g.) and finely divided potassium (1·6 g.) in pure anhydrous benzene (70 c.c.) was heated under reflux on the water-bath for 138 hours with β-6-methoxy-1-naphthylethyl bromide (8·4 g.). The product was treated with ice-water, and the benzene solution separated, ether being used to complete the extraction. The combined extracts were washed with brine, dried, and concentrated. Vacuum distillation of the residue gave unchanged ethyl methyl*cyclo*pentanonecarboxylate (5 g.), b. p. 120—124°/20 mm., unchanged methoxynaphthylethyl bromide (3·5 g.), b. p. 145—155°/0·15 mm., and the desired *keto-ester* (4·8 g.), b. p. above 210°/0·1 mm. The last fraction was redistilled, giving a viscous yellow oil, b. p. 212°/0·2 mm. (*Found: C, 75·6; H, 6·9. $C_{22}H_{26}O_4$ requires C, 74·6; H, 7·35%). The *semicarbazone* crystallised from methyl alcohol in small yellowish plates, m. p. 161—163° (Found: N, 9·8. $C_{23}H_{29}O_4N_3$ requires N, 10·2%).

7-*Methoxy*-1′-*methyl*-1 : 2-cyclo*pentenophenanthrene*.—The foregoing keto-ester (1·9 g.) was boiled for 6 hours with sulphuric acid (37 c.c.) (equal volumes of concentrated acid and water). After cooling, ice was added, and the dark resinous product digested repeatedly with small quantities of ether. The washed and dried ethereal extract was distilled in a vacuum. The product, b. p. 150—160°/0·1 mm., crystallised from alcohol containing a little acetic acid in colourless plates (0·45 g.), m. p. 93—95°. This was treated, in alcoholic solution, with an equal weight of *s*.-trinitrobenzene. The resulting *complex* formed orange-yellow needles of constant m. p. 129° (Found: C, 62·9; H, 4·4. $C_{19}H_{18}O,C_6H_3O_6N_3$ requires C, 63·1; H, 4·5%). 7-*Methoxy*-1′-*methyl*-1 : 2-cyclo*pentenophenanthrene*, regenerated by removal of the trinitrobenzene (stannous chloride), followed by high vacuum sublimation, formed colourless plates (from alcohol), m. p. 97·5—98° (Found: C, 86·7; H, 6·8; OMe, 12·1. $C_{19}H_{18}O$ requires C, 87·0; H, 6·9; OMe, 11·8%).

Ethyl 2-(β-6′-*Methoxy*-1′-*naphthylethyl*)-4-*methyl*cyclo*pentanone*-2-*carboxylate*.—This was prepared exactly as described for the 5-methyl compound from a solution of potassium (1·5 g.) in ethyl 4-methyl*cyclo*pentanone-2-carboxylate (Dieckmann, *Annalen*, 1901, **317**, 78) (7·8 g.) and benzene (80 c.c.), followed by addition of methoxynaphthylethyl bromide (8·6 g.), boiling being continued for 66 hours. The desired *keto-ester* (6·1 g.) formed a pale yellow, very viscous liquid, b. p. about 205°/0·05—0·1 mm. (*Found: C, 74·2; H, 7·4. $C_{22}H_{26}O_4$ requires C, 74·6; H, 7·35%).

7-*Methoxy*-2′-*methyl*-1 : 2-cyclo*pentenophenanthrene*.—Cyclisation and dehydrogenation was effected by 5 hours' boiling of the above keto-ester (1·6 g.) with sulphuric acid (1 : 1 by volume; 38 c.c.). After isolation as described in the preceding case, the product (1 g. of distillate, b. p. 160—170°/0·1—0·2 mm.) was dissolved in hot alcohol (15 c.c.). The leaflets which separated on cooling were rapidly collected (long standing gave also some granular impurity) and had m. p. 134—135°. Purification through the *s*.-trinitrobenzene complex was effected in the customary way, the resulting 7-*methoxy*-2′-*methyl*-1 : 2-cyclo*pentenophenanthrene* forming small

colourless needles (from alcohol), m. p. 136·5—137·5° (Found : C, 87·2; H, 7·0; OMe, 11·4. $C_{19}H_{18}O$ requires C, 87·0; H, 6·9; OMe, 11·8%). The s.-*trinitrobenzene* complex crystallised from alcohol in microscopic golden-orange needles, m. p. 132—133°, depressed by the analogous derivative of 7-methoxy-3′-methyl-1 : 2-*cyclo*pentenophenanthrene (Found : C, 63·0; H, 4·4. $C_{19}H_{18}O,C_6H_3O_6N_3$ requires C, 63·1; H, 4·5%).

Synthesis of 7-*Methoxy*-3′-*methyl*-1 : 2-cyclo*pentenophenanthrene*.

1-(β-6′-*Methoxy*-1′-*naphthylethyl*)-2 : 5-*dimethyl*-Δ^1-cyclo*pentene*.—2 : 5-Dimethyl*cyclo*pentanone (Cornubert and Borrel, *loc. cit.*) (5 g.) was added to an ice-cold Grignard solution prepared from β-6-methoxy-1-naphthylethyl bromide (8·8 g.), magnesium turnings (0·8 g.), and anhydrous ether (25 c.c.). After an hour at room temperature and then ½ hour's boiling, the product was decomposed with ice and ammonium chloride, and the ethereal solution washed, dried, and distilled. There were obtained unchanged ketone (2·8 g.), b. p. 60°/20 mm. (semicarbazone, m. p. 189—191°), the desired carbinol (3 g.), b. p. 160—200°/0·5 mm., and a residue of dimethoxydinaphthylbutane (2 g.) (compare Part III). The crude carbinol was heated for an hour at 160° with potassium hydrogen sulphate (4·5 g.), and the product isolated and distilled (2·2 g.; b. p. 164—169°/0·4 mm.). The *picrate* of 1-(β-6′-*methoxy*-1′-*naphthylethyl*)-2 : 5-*dimethyl*-Δ^1-cyclo*pentene* formed an orange-yellow crystalline powder, m. p. 80—82° (Found : C, 60·8; H, 5·3. $C_{20}H_{24}O,C_6H_3O_7N_3$ requires C, 61·3; H, 5·35%).

7-*Methoxy*-1 : 3′-*dimethyl*-1 : 2 : 3 : 4-*tetrahydro*-1 : 2-cyclo*pentenophenanthrene*.—Cyclisation of the olefin (2 g.) in carbon disulphide (20 c.c.) by aluminium chloride (2 g.) was complete at 0° in 5 hours. The product, b. p. 175°/0·2 mm., gave a *picrate*, which crystallised from methyl alcohol in orange-red needles, m. p. 87—88° (Found : C, 60·2; H, 5·0; OMe, 6·1. $C_{20}H_{24}O,C_6H_3O_7N_3$ requires C, 61·3; H, 5·35; OMe, 6·1%). This picrate had a strong tendency to dissociate when recrystallised, which probably accounts for the low value for carbon. The s.-*trinitrobenzene* complex, prepared from the liquid methoxy-compound regenerated from the picrate, formed small orange crystals (from alcohol), m. p. 98° (Found : C, 63·2; H, 5·6. $C_{20}H_{24}O,C_6H_3O_6N_3$ requires C, 63·25; H, 5·5%).

7-*Methoxy*-3′-*methyl*-1 : 2-cyclo*pentenophenanthrene* (VIII).—The pure 1 : 3′-dimethyltetrahydro-compound (0·15 g.) was heated with selenium (0·3 g.) at 300—310° for 15 hours. Purification of the crystalline product was effected as with the *cyclo*pentenophenanthrene derivatives obtained from the hormones. 7-*Methoxy*-3′-*methyl*-1 : 2-cyclo*pentenophenanthrene* formed small colourless plates, m. p. 146·5—148·5° (Found : C, 87·1; H, 6·7. $C_{19}H_{18}O$ requires C, 87·0; H, 6·9%). The mixed m. p. with the methoxydimethyl*cyclo*pentenophenanthrene prepared from oestrone was 143·5—144·5° (to a cloudy liquid). The s.-*trinitrobenzene* complex of this synthetic 3′-methyl compound had m. p. 135·5—136·5° (Found : C, 62·7; H, 4·5. $C_{19}H_{18}O,C_6H_3O_6N_3$ requires C, 63·1; H, 4·5%).

Synthesis of 7-*Methoxy*-3′ : 3′-*dimethyl*-1 : 2-cyclo*pentenophenanthrene* (V).

2 : 2-Dimethyl*cyclo*pentanone was prepared from 2-methyl*cyclo*pentanone (Cornubert and Borrel, *loc. cit.*) by methylation with sodamide and methyl iodide (Haller and Cornubert, *Compt. rend.*, 1914, **158**, 300). In order to obtain good results it was necessary to proceed as follows : Finely powdered sodamide (12 g. = 50% excess) was added gradually to 2-methyl*cyclo*pentanone (20 g.) diluted with anhydrous ether (200 c.c.). After evolution of ammonia had ceased, methyl iodide (42 g.) was added dropwise; the mixture then boiled spontaneously and continued to do so for ½ hour. The reaction was completed by 3 hours' heating on the water-bath. During the whole of this series of operations the mixture was stirred vigorously. The resulting 2 : 2-dimethyl*cyclo*pentanone (16·5 g.) had b. p. 143—145°, and gave a semicarbazone, m. p. 189—191° (lit., 193°). Haller and Cornubert (*loc. cit.*) state that methylation of this ketone gives a mixture of 2 : 2 : 5-trimethyl- (b. p. 151—152°) and 2 : 2 : 5 : 5-tetramethyl- (b. p. 155—156°) *cyclo*pentanones, which they claim to have separated by fractional distillation. These b. p.'s are so close that separation must be very difficult. By methylation of the dimethyl ketone in the manner described above we obtained a product, b. p. 150—152°, which gave (in poor yield) a semicarbazone which crystallised in well-formed needles and had constant m. p. 163·5—164·5° (the m. p. given in the literature for 2 : 2 : 5-trimethyl*cyclo*pentanone semicarbazone is 150—151°). However, analysis showed that our semicarbazone was not a pure compound, although probably consisting mostly of the semicarbazone of the trimethyl ketone (Found : C, 57·85; H, 9·1; N, 23·0. $C_9H_{17}ON_3$ requires C, 59·0; H, 9·4; N, 22·9%).

In order to avoid complications due to the heterogeneous nature of our ketone we employed

another route to 2 : 2 : 5-trimethyl*cyclo*pentanone. For this purpose, 2 : 2-dimethyl*cyclo*pentanone (22 g.) was condensed with ethyl oxalate (28·5 g.) in presence of sodium ethoxide (4·6 g. of sodium in 60 c.c. of alcohol) exactly as described by Kötz and Michels (*Annalen*, 1906, **350**, 210; 1908, **358**, 198) in an analogous case. The resulting *ethyl* 2 : 2-*dimethyl*cyclo*pentanone*-5-*glyoxylate* (27 g.), which was not converted by heat into the β-keto-ester, was purified by solution in cold dilute alkali, extraction of neutral material with ether, and then liberation of the free diketo-ester. It formed a colourless liquid, b. p. 141°/12 mm., which gave a brownish-red colour with ferric chloride (*Found : C, 61·3; H, 7·4. $C_{11}H_{16}O_4$ requires C, 62·2; H, 7·6%). This ester was hydrolysed by heating for an hour with an equal weight of potassium hydroxide in aqueous alcoholic solution. 2 : 2-*Dimethyl*cyclo*pentanone*-5-*glyoxylic acid*, precipitated by hydrochloric acid, was recrystallised from water, ligroin, and finally water; it then formed colourless plates, m. p. 75—76°, and gave an intense brownish colour with ferric chloride (*Found : C, 58·4; H, 6·65. $C_9H_{12}O_4$ requires C, 58·1; H, 6·6%).

For methylation, the potassio-compound of the diketo-ester (from 5·3 g. of potassium and 24 g. of ester in 90 c.c. of benzene) was boiled for 5 hours with methyl iodide (21·5 g.), and then kept over-night at room temperature. *Ethyl* 2 : 2 : 5-*trimethyl*cyclo*pentanone*-5-*glyoxylate* (12 g.), isolated in the usual way, had b. p. 135°/10 mm. (*Found : C, 63·3; H, 8·0. $C_{12}H_{18}O_4$ requires C, 63·7; H, 8·0%).

This ester (9 g.) was boiled for 2 hours with concentrated hydrochloric acid (30 c.c.) and water (30 c.c.). The cold solution was saturated with ammonium chloride and extracted with ether. The resulting 2 : 2 : 5-trimethyl*cyclo*pentanone (3·6 g.) gave a semicarbazone, m. p. 149—150° (Found : C, 59·1; H, 9·05; N, 22·95%).

An ice-cold Grignard solution prepared from β-6-methoxy-1-naphthylethyl bromide (6·8 g.) was treated with 2 : 2 : 5-trimethyl*cyclo*pentanone (3·1 g.), and the whole boiled for 20 hours. The product was worked up in the usual way. The very incomplete reaction, presumably owing to enolisation of the ketone, led to the isolation of 5-*ethylnerolin* (3·2 g.; b. p. 120—130°/0·5 mm., 165°/10 mm.), which was purified through its orange *picrate*, m. p. 83—84° (Found : N, 9·7. $C_{13}H_{14}O,C_6H_3O_7N_3$ requires N, 10·1%), and then formed colourless prismatic needles, m. p. 54—55° (Found : C, 84·0; H, 7·55. $C_{13}H_{14}O$ requires C, 83·8; H, 7·6%).

The crude carbinol fraction (0·7 g.; b. p. above 190°/0·3 mm.) arising from this Grignard condensation was dehydrated † with potassium hydrogen sulphate (1 g.) at 160°, extracted with carbon disulphide (10 c.c.), and the ice-cold solution treated with anhydrous aluminium chloride (0·7 g.). After remaining over-night at 2—3°, the clear solution was poured off and shaken with water. The pale yellow gum remaining after removal of carbon disulphide was heated with selenium (0·1 g.) at 300—320° for 13 hours. The product was extracted with ether and distilled at 0·2 mm. from an air-bath at 200°. The distillate (50 mg.) was treated with an alcoholic solution of *s.*-trinitrobenzene (30 mg.). After being twice recrystallised from alcohol, the *s.*-trinitrobenzene complex formed golden-orange needles, m. p. 170—171·5°, not depressed by the analogous complex of the 7-methoxy-3′ : 3′-dimethyl-1 : 2-*cyclo*pentenophenanthrene prepared from oestrone. By reduction with stannous chloride there was obtained the dimethyl compound (V), which crystallised from methyl alcohol in colourless plates, m. p. 165—165·5°, not depressed by the specimen prepared from oestrone (yield, about 2 mg.). This specimen, and the one from oestrone, gave an identical series of colour changes with concentrated sulphuric acid.

Demethylation of 7-*Methoxy*-1-*methyl*-1 : 2 : 3 : 4-*tetrahydro*-1 : 2-cyclo*pentenophenanthrene.*

The hydroxy-compound corresponding to this methoxy-compound, the synthesis of which was described in Part III, was required in order to investigate its oestrogenic activity. Attempts to effect demethylation by acid reagents led to dark coloured tars. By using the following procedure the pure hydroxy-compound was readily obtained, although demethylation was incomplete : The methoxy-compound (0·8 g.) was heated with sodium ethoxide (0·8 g. of sodium in 16 c.c. of alcohol) at 190—195° for 20 hours. The solution, after dilution with water, was extracted with ether. The clear aqueous solution was then acidified, and the phenol extracted with ether. The product (0·1 g.) was treated with benzoyl chloride in pyridine,

† We were, of course, fully aware of the possibility of methyl migration during the dehydration of the carbinol arising from the interaction of the trimethyl*cyclo*pentanone with the methoxynaphthylethylmagnesium bromide. However, although one of the carbon atoms contiguous to the carbinol group is quaternary, the other is tertiary, and we were inclined to the belief that this would tend to promote normal dehydration, without migration. This view was justified by the results.

and the *benzoate* sublimed in a high vacuum at 170°. After recrystallisation from aqueous alcohol it formed colourless cubic crystals, m. p. 141—141·5° (Found : C, 84·05; H, 6·8. $C_{25}H_{24}O_2$ requires C, 84·2; H, 6·8%). The " non-acidic " fraction obtained from the original ethereal extract still contained a considerable amount of the phenol, of which 0·17 g. was isolated by trituration with light petroleum. The resulting 7-*hydroxy*-1-*methyl*-1 : 2 : 3 : 4-*tetrahydro*-1 : 2-cyclo*pentenophenanthrene* crystallised from light petroleum in colourless nodules, m. p. 131·5—132°, the same compound being obtained by hydrolysis of the benzoate described above (Found : C, 85·6; H, 7·9. $C_{18}H_{20}O$ requires C, 85·7; H, 8·0%). The liquors from the isolation of this phenol contained chiefly unaltered methoxy-compound, identified by conversion into its characteristic *s*.-trinitrobenzene complex.

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