THE PREPARATION AND PROGESTATIONAL ACTIVITY OF SOME ALKYLATED ETHISTERONES

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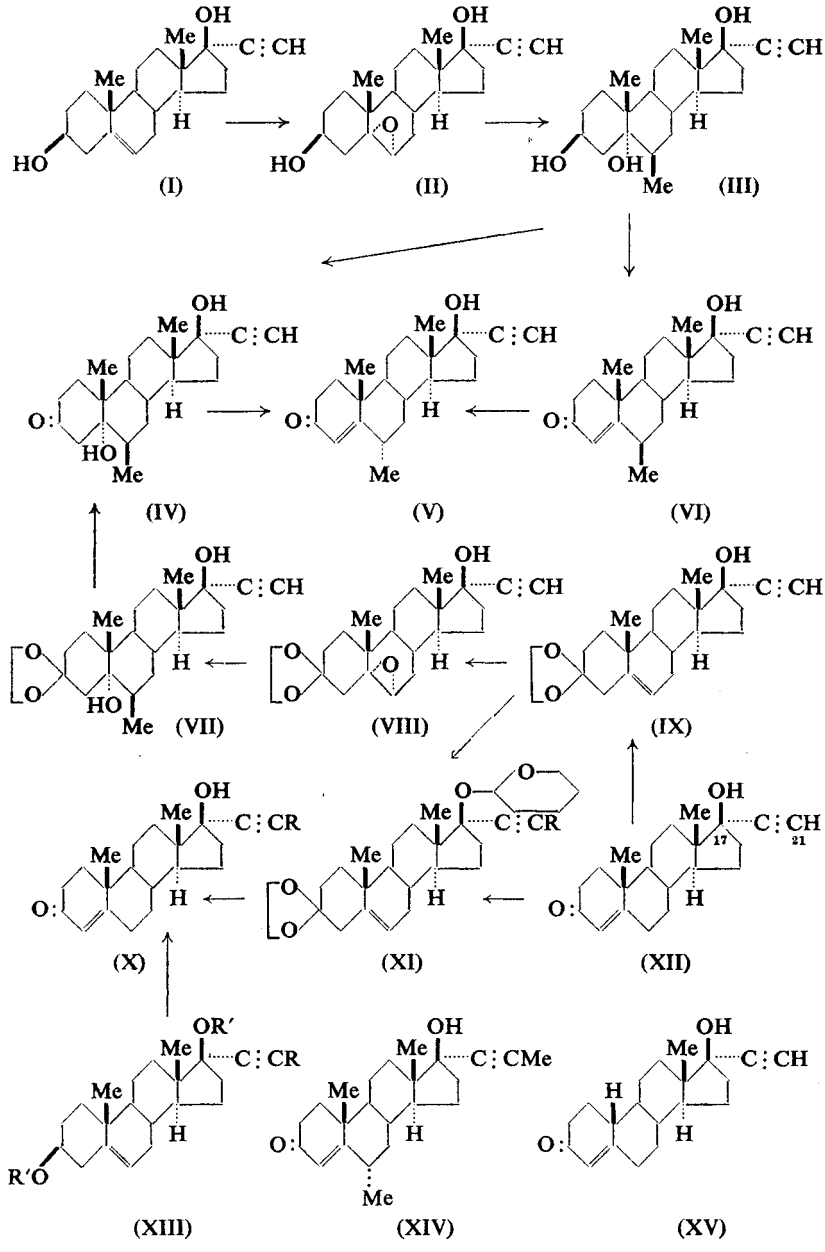
The preparation and progestational activity of some 6-alkyl, 21-alkyl and 6:21-dialkyl derivatives of ethisterone are described. 6α:21-Dimethylethisterone (XIV), the most potent compound of the series, given by mouth proved to have approximately twelve times the activity of ethisterone in the Clauberg test.

The present studies were initiated in 1954 and had as their object the preparation of an orally-acting progestational agent more potent than ethisterone (XII). At that time the literature contained several references to unsuccessful attempts to achieve the same objective. In addition, the paper by Djerassi, Miramontes, Rosenkranz and Sondheimer[1] had just appeared describing the partial synthesis of 19-norethisterone (XV), which had several times the activity of ethisterone in the Clauberg test. The 19-nor steroid type (XV), however, appeared unattractive for development as its preparation involved Birch reduction of oestradiol methyl ether, a raw material which could hardly be regarded as abundant and cheap.

At that time the interests of our research group had been focussed on speculations concerning the possible role of methylated steroids, and in particular 4:4-dimethyl steroids, as biogenetic precursors of the steroid hormones[2]. It seemed desirable therefore to consider the methylated derivatives of ethisterone. Selection of 6-methylethisterone for prior study stemmed largely from biogenetic considerations. The liver was known to deactivate steroid hormones in several ways including β-hydroxylation at C(6)[3]. By blocking this centre with a methyl group we hoped to prevent such oxidation and thereby enhance biological activity by the oral route.

Preparation of the isomeric 6α- and 6β-methylethisterones was achieved in the following way[4]. Ethynylandrostenediol (I) was converted into the 5α:6α-epoxide (II), which passed smoothly into 17α-ethynyl-6β-methylandrostane-3β:5α:17β-triol (III) on reaction with methyl magnesium iodide. Oppenauer oxidation of the latter compound afforded 6β-methylethisterone (VI), which was readily isomerised to 6α-methylethisterone (V) by alkaline or acidic reagents. The same compound (V) was additionally obtained by oxidising the triol (III) to the 3-oxo derivative (IV), which was converted into the desired product by dehydration and epimerisation.

An alternative route to 6α-methylethisterone (V) employing ethisterone (XII) as starting material was also developed[5]. In this process ethisterone (XII) was converted into the 3:3-ethylenedioxy-derivative (ethylene ketal)



(IX), a transformation accompanied by migration of the 4:5-ethylenic linkage into the 5:6-position. Treatment of this compound with monoperphthalic acid gave the 5α:6α-epoxide (VIII), which passed into the 6β-methyl-5α-hydroxy steroid (VII) on reaction with methylmagnesium iodide. This product (VII) readily lost the ketal group on contact with

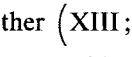
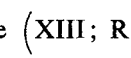
very dilute acid to give the intermediate (IV), which was transformed into 6α-methylethisterone (V) as before. 6β-Ethylethisterone was prepared in essentially the same way (see exptl.).

Biological study of 6β-methylethisterone (VI) proved disappointing, the compound being only about one-third as potent as ethisterone. The 6β-ethyl-derivative was virtually devoid of activity. 6α-Methylethisterone (V), in contrast, was found to be approximately seven times as active as ethisterone in the Clauberg test.

The marked superiority of 6α- over 6β-methylethisterone as a progestational agent is difficult to interpret in terms of increased resistance to 6β-hydroxylation by the liver[3], and it seems likely that other factors are involved. This view is strengthened by the observation that similar quantitative differences obtain with other 6-methylated steroid hormone pairs prepared in our laboratories. The role played by the alkyl group in modifying biological activity, however; remains to be elucidated.

We next turned our attention to the alkylation of ethisterone at C(21). The reasons which prompted this choice stemmed from the structural analogy between ethisterone and methyltestosterone, which could both be regarded as 17-substituted testosterone derivatives.

Now stepwise increase in the alkyl chain attached to C(17) in 17α-methyltestosterone changes markedly the biological properties of this orally active androgen. In analogy stepwise increase in the alkynyl chain attached to C(17) in 17α-ethynyltestosterone (ethisterone) to give 21-alkyl derivatives was thought worthy of study.

Preparation of the 21-alkyl derivatives of ethisterone (X) was accomplished by the following route[6]. Ethynylandrostenediol (XIII; R = R′ = H) was treated with 2:3-dihydropyran and phosphorus oxychloride to give the bis-tetrahydropyranyl ether (XIII; R = H, R′ = ). This operation proved necessary to avoid alkylation of the hydroxyl groups during the subsequent operation. The bis-tetrahydropyranyl ether was then converted into the C(21)-lithium derivative by treatment with lithamide in liquid ammonia, which was then alkylated with an alkyl halide to give the derivative (XIII; R = alkyl, R′ = ). The product obtained by removing the protecting groups was submitted to Oppenauer oxidation to give the ethisterone homologue (X; R = alkyl). Alternatively, the 3:3-ethylenedioxy-derivative of ethisterone (IX) was treated with 2:3-dihydropyran to give the product (XI; R = H), which was alkylated as before yielding the intermediate (XI; R = alkyl). Treatment of this compound with dilute acid furnished the 21-alkyl-ethisterone (X; R = alkyl).

Bioassay of the ethisterone homologues (X; where R = Me, Et, *n*-Pr and *n*-Bu, respectively) revealed an interesting gradation of properties.

21-Methylethisterone was found to be about three times as active as ethisterone. Increase in the size of the alkyl substituent led successively to a decrease of activity, the *n*-butyl derivative being less active than ethisterone itself.

Having established that the biological activity of ethisterone was enhanced by (i) a 6α-methyl substituent and (ii) an alkyl group (Me > Et > Pr) at C(21), it was clearly essential to determine whether these effects would prove additive. We therefore synthesised the 21-methyl and 21-ethyl derivatives[6] of 6α-methyl-ethisterone by procedures based on those described above. Biological study of these compounds revealed that the effects were, broadly speaking, additive. Thus 6α:21-dimethyl-ethisterone (XIV) proved to be approximately twelve times and 21-ethyl-6α-methylethisterone nine times more active than ethisterone in the Clauberg test. 6α:21-Dimethylethisterone (XIV) is thus the most potent orally active progestational agent based upon 10:13-dimethylperhydro-*cyclo*pentenophenanthrene that has yet been reported. Clinical studies are in progress.

Experimental

Melting points are uncorrected.

Preparation of 6β-ethylethisterone (with Miss D. Wedlake, B.Sc.).—3β-Acetoxy-5α:6α-epoxy-17α-ethynylandrostan-17β-ol[4] (13·1 g.) in benzene (500 ml.) was added rapidly to a stirred Grignard reagent prepared from magnesium (7·2 g.), ethyl iodide (42 g.) and ether (320 ml.). The mixture was distilled until the vapour temperature reached 78° when more benzene (300 ml.) was added. After heating under reflux for 5 hours the mixture was cooled, treated with a slight excess of dilute sulphuric acid, and the organic layer washed with water and dried. The residue obtained by removal of the solvent was crystallised from aqueous methanol to give *6β-ethyl-17α-ethynylandrostane-3β:5α:17β-triol*, needles, m.p. 143° to 145° $[\alpha]_D^{20}$ − 45° (*c*, 1·0 in pyridine). Found: C, 73·6; H, 10·3. $C_{23}H_{36}O_3$ requires C, 73·0; H, 10·2 per cent.

The foregoing triol (4·5 g.) in toluene (250 ml.) and *cyclo*hexanone (110 ml.) was treated with aluminium *iso*propoxide (2·5 g.) in toluene (12 ml.). The mixture was heated under reflux for 40 minutes, cooled, washed with dilute sulphuric acid, then with water, and the solvents removed by steam distillation. The product was purified from methanol to give *6β-ethylethisterone*, rods, m.p. 260° to 264°, $[\alpha]_D^{20}$ + 9° (*c*, 0·7 in pyridine). Found: C, 80·9; H, 9·4. $C_{23}H_{32}O_2$ requires C, 81·1; H, 9·5 per cent.

Estimation of Progestational Activity

McPhail's modification[7] of the Clauberg test was employed. Immature female rabbits weighing between 800 to 1,200 g. were sensitised with a total dose of 15 μg. of oestrone in 0·6 ml. of a mixture of ethyl oleate (20 per cent) with arachis oil, given intramuscularly, in three equal amounts, on days one, three and five of the test.

Ethisterone and the test compounds were given orally in four equal parts on days seven, eight, nine and ten. Doses were given suspended in 5 ml. of a mucilage of acacia (5 per cent). The animals were killed on day eleven and frozen sections of uteri of 20μ thickness were prepared, stained with haematoxylin and examined for progestational response. Projection

drawings of the uteri were prepared and the responses estimated by measuring the fraction of endometrium occupied by glandular tissue. In addition, McPhail's method of scoring was used and good agreement between the methods was found. Potencies were however calculated using the first method which being susceptible of repeated check measurements was considered to be less liable to error from bias.

The relative activities of four 21-alkyl derivatives of ethisterone were estimated in a multiple four point assay, using fifty rabbits. Table I records the results.

TABLE I

MEAN PROGESTATIONAL RESPONSES IN FIVE RABBITS PER DOSE AFTER ORAL ADMINISTRATION OF FOUR 21-ALKYL DERIVATIVES OF ETHISTERONE

Compound	Response per cent glandular tissue		Approximate relative potency
	Dose 2·5 mg./kg.	Dose 7·5 mg./kg.	
Ethisterone	54·4	67·0	1·0
21-Methylethisterone	66·4	70·8	3·0
21-Ethylethisterone	58·2	69·3	1·6
21-Propylethisterone	56·0	64·6	1·0
21-Butylethisterone	50·3	58·6	0·5

The 6α-methyl-, 6α:21-dimethyl-, and 6α-methyl-21-ethyl-derivatives were also compared in a multiple six point assay, using sixty rabbits. Table II records the results.

TABLE II

MEAN PROGESTATIONAL RESPONSES IN FIVE RABBITS PER DOSE AFTER ORAL ADMINISTRATION OF VARYING AMOUNTS OF 6α-METHYL, 6α:21-DIMETHYL AND 6α-METHYL-21-ETHYL DERIVATIVES OF ETHISTERONE

Compound	Dose mg./kg.	Response		Relative potency (P = 0·95)
		McPhail	Glandular tissue per cent	
Ethisterone	2·5	1·16	45·8	
	5·0	1·82	54·8	1·0
	10·0	2·54	68·4	
6α-Methylethisterone	0·3	0·76	38·2	
	0·6	1·60	50·8	6·54
	1·2	2·76	66·8	(4·84–8·70)
6α:21-Dimethylethisterone ..	0·3	1·62	53·4	
	0·6	2·36	59·6	11·5
	1·2	3·20	73·4	(8·67–15·5)
6α-Methyl-21-ethyl ethisterone	0·3	1·12	43·8	
	0·6	1·86	57·6	9·24
	1·2	3·26	73·2	(6·93–12·4)

REFERENCES

1. Djerassi, Miramontes, Rosenkranz and Sondheimer, *J. Amer. chem. Soc.*, 1954, **76**, 4092.
2. Cf. Cooley, Ellis and Petrow, *J. chem. Soc.*, 1955, 2998; Adams, Patel, Petrow, Stuart-Webb and Sturgeon, *ibid.*, 1956, 4490.
3. See, for example, Miller and Axelrod, *Metabolism*, 1954, **3**, 438.

4. B.P. Provisional Specifications Nos. 15889/55, 16645/55, 17799/55, 18118/55. Burn, Ellis, Petrow, Stuart-Webb and Williamson, in the press; Ackroyd, Adams, Ellis, Petrow and Stuart-Webb, in the press.
5. B.P. Provisional Specification No. 9378/56. Cooley, Ellis, Kirk and Petrow, in the press.
6. B.P. Provisional Specification No. 2820/57.
7. McPhail, *J. Physiol.*, 1935, **83**, 145.

DISCUSSION

The paper was presented by DR. V. PETROW.

THE CHAIRMAN. Preliminary clinical tests confirmed that 6α-methyl ethisterone was at least three times as active as ethisterone. Clinical confirmation about the 6α:21-dimethyl compound was not yet available.

DR. L. M. ATHERDEN (Sunderland) noted that ethisterone possessed a double bond in the 4:5 position which would be hydrogenated in the liver. Would the 6α-methyl compound be more active because the hydrogenation would be hindered?

DR. J. B. STENLAKE (Glasgow). Had the study of the 6α-alkyl substances been carried any further?

DR. G. BROWNLEE (London). Had the substances any androgenic activity?

DR. V. PETROW replied that he knew of no published work describing the fate of ethisterone in the body. The study of the 6α-alkyl substances had not been continued because of the drop in activity.

DR. DAVID added that the substances possessed no androgenic properties; at very high and unphysiological doses the 6α-methyl compound showed a very slight anabolic effect.