THE BIOSYNTHESIS OF 23,24-DINOR-5-CHOLENE-3β,20-DIOL AND 23,24-DINOR-5-CHOLENE-3β,21-DIOL.

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ABSTRACT

An alternative pathway for steroidogenesis, via a sesterterpene, has been proposed. This communication presents evidence that two of the proposed compounds with the 23,24-dinorcholane carbocyclic system, 23,24-dinor-5-cholene-3β,20-diol and 23,24-dinor-5-cholene-3β,21-diol, can be biosynthesised from sodium [^{3}H]acetate in a bovine adrenal preparation.

The possibility of steroidogenesis through a C_{25} terpene (sesterterpene) pathway, with compounds based on the 23,24-dinorcholane carbocyclic system as intermediates, has been discussed (1). The conversion of 23,24-dinor-5-cholen-3β-ol (I) to 23,24-dinor-5-cholene-3β,21-diol (II) and 23,24-dinor-5-cholene-3β,20-diol (III), and of the latter to steroid hormones was demonstrated (1). This communication describes studies on the biosynthesis of these materials from radioactive acetate.

EXPERIMENTAL

Sodium [^{3}H]acetate (specific radioactivity 500 Ci/mol) and [7α-^{3}H]cholesterol (specific radioactivity 500 Ci/mol) were purchased from the Radiochemical Centre.

Incubations were carried out on bovine adrenal slices (approx. 10 g. of tissue) at 37°C for 5 hr. in 0.1 M phosphate buffer, pH 7.4 (2), containing nicotinamide (30 mmol/l), magnesium chloride (4 mmol/l), and NAD (250 μmol/l). Each incubation flask contained 23,24-dinor-5-cholen-3β-ol (1 mg.), 23,24-dinor-5-cholene-3β,20-diol (1 mg.) and 23,24-dinor-5-cholene-3β,21-diol (1 mg.), as traps for radioactivity. Steroids were isolated, quantitative measurements made and radioactivity determined as previously described (1), with the exception that no further quantities of carrier steroid were added. T.l.c. solvent systems used were:

toluene-ethyl acetate (A, 9:1,v/v; B, 4:1, v/v; C, 2:1, v/v; D, 3:2, v/v); cyclohexane-acetone (E, 9:1, v/v; F, 4:1, v/v; G, 2:1, v/v; H, 3:2, v/v); cyclohexane-ethyl acetate (J, 3:2, v/v; K, 1:1, v/v); dichloromethane (L); dichloromethane-ethyl acetate (M, 4:1, v/v; N, 2:1, v/v). One further system (O) in which a Kieselgel plate was treated with 2-3% decalin in hexane for 5 min., air dried, then run in methanol saturated with decalin was also used. This system separated cholesterol from 23,24-dinor-5-cholen-3β-ol. 23,24-Dinor-5-cholene-3β,20-diol 3-acetate was converted to 23,24-dinor-5,20-choladien-3β-ol (3) by treating with benzene saturated with hydrogen chloride and dehydrohalogenating with methanolic potassium hydroxide (4).

Where a steroid was identified, it was dissolved in solvent and portions taken for g.l.c., counting of radioactivity, derivative formation and crystallisation. Procedures for acetylation and hydrolysis of steroids were as described by Bush (7).

RESULTS AND DISCUSSION.

Incubations with sodium [^{3}H]acetate gave incorporation of radioactivity into the dihydroxy compounds (II) and (III), but no incorporation into 23,24-dinor-5-cholen-3β-ol (I), (Table 1).

A control experiment in which [^{3}H]acetate was incubated as described above with boiled tissue (tissue heated in a water bath at 100°C for 30 min) in the presence of the steroid traps was carried out. The steroids were isolated and purified, the radioactivity associated with them being readily removed on chromatography.

A second control experiment in which [7α-^{3}H]cholesterol (25 μCi) was incubated with tissue in the presence of traps was carried out. This experiment examined the possibility that the incorporation of radioactivity could arise via cholesterol, or be due to contamination from metabolites of cholesterol formed in the incubation system. A range of radioactive products was formed, but chromatography as the free alcohols and as acetates in systems K, E, B, M, H, D, L, and F removed radioactivity from the dihydroxy compounds (II) and (III). 23,24-Dinor-

5-cholen-3β-ol (I) was chromatographed as the alcohol and acetate in systems K, E, O, J, L, and F, after which there was still some radioactivity associated with it. Carrier steroid was added and the material was crystallised from methyl alcohol, the radioactivity separating in the mother liquors.

A noteworthy point in these incubations is the low recovery of the steroids added as traps from the incubations, only 20% of the added 23,24-dinor-5-cholene-3β,21-diol (II) (mean of four incubations) and 32% of 23,24-dinor-5-cholen-3β-ol (mean of two incubations) being recovered. That this did not represent experimental losses, but was dependent on length of incubation time, was shown by the fact that two incubations of 0.5 hr. and 1 hr. gave 74% and 53% recovery of the diol (II) respectively. It was also demonstrated that solvolysis of the aqueous layer after extraction of free steroids did not add any appreciable quantity to the diol recovered.

Cholesterol from the sodium [^{3}H]acetate incubations was chromatographed as the alcohol and acetate in systems K, E, O, J, L, F, E, K, and F. Carrier cholesterol was added and crystallised from methanol and specific radioactivities measured. The crops were acetylated and further crystallised and specific radioactivities measured. The radioactivity in the cholesterol fractions purified in this way was 0.4 and 0.7 nCi. The radioactivity in 23,24-dinor-5-cholene-3β,20-diol (III) was 5.6 and 17.4 nCi, and in 23,24-dinor-5-cholene-3β,21-diol (II) was 1.6 and 8.6 nCi.

The incorporation of acetate into the diols (II) and (III) and not into 23,24-dinor-5-cholen-3β-ol (I) could mean that the latter is not a necessary intermediate in their biosynthesis. The report of the conversion of 23,24-dinor-5-cholen-3β-ol (I) to the diols (II) and (III) (1) would argue against this. It may be that the 23,24-dinor-5-cholen-3β-ol (I) formed is rapidly converted to the diols.

The anaolgue to lanosterol in the proposed pathway for steroidogenesis (1) would be 23,24,25,26,27-pentanor-8-lanosten-3β-ol (IV). Van Tamelen and his group (5, 6) in their extensive bio-organic studies of terpenoid terminal epoxides have demonstrated the enzymic cyclisation in a liver preparation of synthetic substrates to give this material (IV).

From the results reported here, taken along with the earlier data (1), it appears that the bovine adrenal gland can biosynthesise compounds with the 23,24-dinorcholane carbocyclic system and use them to make steroid hormones by what could be called a sesterterpene pathway.

Further work is in progress to establish whether 23,24-dinor-5-cholen-3β-ol (I) is an intermediate in this pathway and if these compounds can be biosynthesised from mevalonic acid.

REFERENCES

1. Tait, A.D., BIOCHEM.J. 128, 467-470 (1972).

2. Dawson, R.M.C., Elliott, D.C., Elliott, W.H. & Jones, K.M., DATA FOR BIOCHEMICAL RESEARCH, ed. 2, Oxford University Press, London (1969), p. 489.

3. Sondheimer, F. & Mechoulam, R., J. AMER. CHEM. SOC. 79, 5029-5033 (1957).

4. Uskokovic, M., Dorfman, R.I. & Gut, M., J. ORG. CHEM. 23, 1947-1951 (1958).

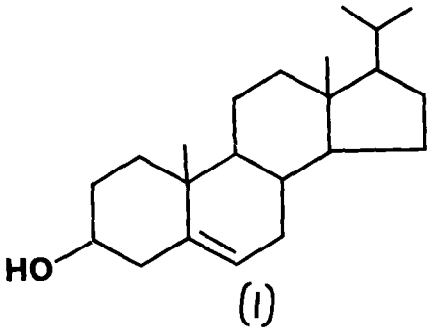
5. Anderson, R.J., Hanslik, R.P., Sharpless, K.B., van Tamelen, E.E. & Clayton, R.B., CHEM. COMMUN., 53-54 (1969).

6. Van Tamelen, E.E. &Freed, J.H., J. AMER. CHEM. SOC. 92, 7206-7207 (1970).

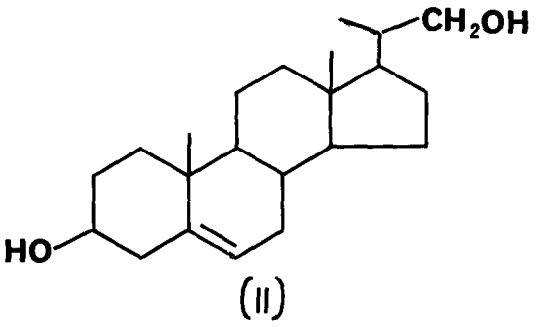
7. Bush, I.E., THE CHROMATOGRAPHY OF STEROIDS, Pergamon Press, London, 1961, pp. 358-371.

Table 1. Incubation of bovine adrenal tissue with sodium [^{3}H]acetate (500 μCi).

Steroid or Reaction	Reaction Product	T.l.c. systems used (nos. in parentheses indicate sequence)	Specific Radioactivity (d.p.m./μmol) Incubation no. 1	2
23,24-Dinor-5-cholene-3β,21-diol		K(1);N(5);H(6);D(7)	6,840	27,600
Acetylation	23,24-Dinor-5-cholene-3β,21-diol diacetate	E(2);B(3);E(4);K(8)	7,500	29,100
Hydrolysis, added carrier steroid, recrystallisation	23,24-Dinor-5-cholene-3β,21-diol		45.1 42.6 42.4	78.1 78.6 78.3
Acetylation and recrystallisation	23,24-Dinor-5-cholene-3β,21-diol diacetate		45.1 45.4 46.2	74.7 74.1 73.1
23,24-Dinor-5-cholene-3β,20-diol		B(1);N(5);H(6);D(7)		
Added carrier steroid, recrystallisation			122.0 123.0 124.0	122.0 121.0 128.0
Acetylation and recrystallisation	23,24-Dinor-5-cholene-3β,20-diol 3-acetate	E(2);D(3);H(4)	129.0 135.0 138.0	136.0 134.0 138.0
Dehydration, added carrier steroid, recrystallisation	23,24-Dinor-5,20-choladien-3β-ol	K	34.1 31.1 33.1	61.8 60.7 59.7
Acetylation and recrystallisation	23,24-Dinor-5,20-choladien-3β-ol acetate		34.6 33.3 32.8	58.5 58.3 57.3



(I)



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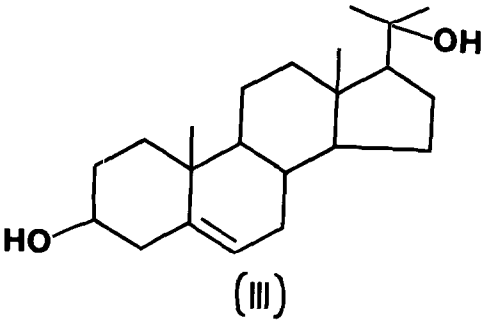

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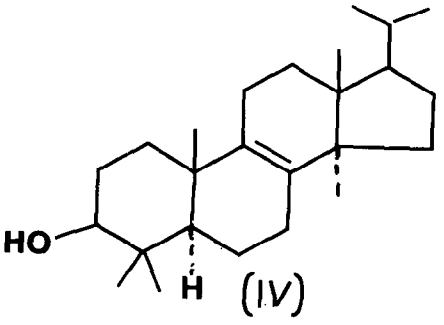

(IV)