The Conversion of 23,24-Dinor- $\text{chol-5-en-3}\beta\text{-ol}$ into Steroid Hormones

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It is widely accepted that cholesterol is the precursor of the steroid hormones. However, there are reports suggesting that there could be some other pathway not involving cholesterol (Heard *et al.*, 1956; Hall, 1964; Shimizu & Gut, 1965).

The biosynthetic pathway through cholesterol involves the triterpenes (six isoprene units) squalene and lanosterol. I have considered the theoretical possibility of steroidogenesis through a C_{25} terpene (five isoprene units) that, on cyclization, gives a compound analogous to lanosterol but with five carbon atoms less on the side chain. This, after demethylation and double-bond migration, would give a compound analogous to cholesterol but with five carbon atoms less, i.e. 23,24-dinor- $\text{chol-5-en-3}\beta\text{-ol}$ (I) (Blickenstaff, 1960; Dusza & Bergmann, 1960).

To test this hypothesis, it was decided to ascertain whether an endocrine tissue could metabolize and biosynthesize this material. This communication reports results on the first of these objectives, i.e. the metabolism of 23,24-dinor- $\text{chol-5-en-3}\beta\text{-ol}$.

Experimental

23,24-Dinor-[$7\alpha\text{-}^3\text{H}$]chol-5-en- $3\beta\text{-ol}$ (I) (specific radioactivity approx. 1.0 Ci/mol) was synthesized from [$7\alpha\text{-}^3\text{H}$]pregnenolone ($3\beta\text{-hydroxy}[7\alpha\text{-}^3\text{H}]\text{-pregn-5-en-20-one}$) via the Wittig reaction with methylenetriphenylphosphorane, giving 23,24-dinor-[$7\alpha\text{-}^3\text{H}$]chola-5,20-dien- $3\beta\text{-ol}$ (Sondheimer & Mechoulam, 1957). The latter was selectively hydrogenated at the 20-double bond by using the soluble catalyst tris(triphenylphosphine)rhodium chloride (Birch & Walker, 1966), giving 23,24-dinor-[$7\alpha\text{-}^3\text{H}$]chol-5-en- $3\beta\text{-ol}$. 23,24-Dinor-[$7\alpha\text{-}^3\text{H}$]chol-5-ene- $3\beta,20\text{-diol}$ (specific radioactivity approx. 5.0 Ci/mol) was synthesized from [$7\alpha\text{-}^3\text{H}$]pregnenol-

one by the Grignard reaction with methylmagnesium iodide (Marker *et al.*, 1942). The products of the reactions were purified as the free steroids and as the acetates by t.l.c. At each purification step, unlabelled starting material was added to the reaction product and the compounds were separated by t.l.c. to preclude the possibility of any labelled pregnenolone remaining in the preparation. The purity of the radioactive compounds thus prepared was checked and was satisfactory by the criteria of isotope-dilution analysis (Chaudhuri & Gut, 1969).

Incubations were carried out on slices and homogenates (approx. 5 g of tissue) in gassed ($\text{O}_2 + \text{CO}_2$, 95:5) Krebs-Ringer bicarbonate buffer, pH 7.4 (DeLuca & Cohen, 1964) containing NADPH ($50\mu\text{mol/l}$). Homogenates were prepared by first slicing the tissue and then homogenizing in a little of the buffer in a manually operated Jencons homogenizer, after which more of the buffer was added (final volume 30 ml). Steroids were extracted with hot acetone (200 ml) and ethanol (200 ml), and the extracts were concentrated under vacuum. Ether (300 ml) was added, and the ethereal solution was washed with water and 0.5 M-KOH ($3 \times 40\text{ml}$) and then water until the washings were neutral. The ethereal solution was evaporated to dryness under vacuum.

The individual radioactive steroids were isolated and, after the addition of carrier steroid ($100\mu\text{g}$), purified by extensive t.l.c. and formation of derivatives. T.l.c. was carried out on 0.25 mm-thick layers of Kieselgel HF 254/366 (Merck), the following solvent systems being used: benzene-ethyl acetate (A, 50:1, v/v; B, 9:1, v/v; C, 3:2, v/v); cyclohexane-acetone (D, 9:1, v/v; E, 7:3, v/v; F, 3:2, v/v); chloroform-acetone (G, 19:1, v/v; H, 9:1, v/v); dichloromethane (J); dichloromethane-ethyl acetate (K, 9:1, v/v). Quantitative measurement of steroids was by g.l.c. on a 1% XE-60 column, a flame ionization detector being used. The labelled steroids were further characterized by adding unlabelled steroids (approx. 20 mg) to a portion of the material and crystallizing to constant specific radioactivity (at least three consecutive crystallizations agreeing to within $\pm 5\%$).

Radioactivity was determined on a Packard Tri-Carb scintillation counter, the efficiency for ^3H being 32%. Portions of the sample were taken such that the count rate was at least twice that of the background.

Table 1. *Incubation of bovine adrenal tissue with 23,24-dinor-[7 α -³H]chol-5-ene-3 β -ol (3 μ Ci)*

Incubations were carried out at 37°C for 2.5h. For general instructions see the Experimental section. Where a steroid was identified, it was dissolved in solvent and portions were taken for g.l.c., counting of radioactivity, derivative formation and crystallization. Procedures for reduction, oxidation, acetylation and hydrolysis of steroids were as described by Bush (1961).

Steroid or reaction	Reaction product	T.l.c. systems used (nos. in parentheses indicate sequence)	Specific radioactivity (d.p.m./ μ mol)			Total radioactivity in metabolite (nCi)		
			Incubation no.			Incubation no.		
			1	2	3	1	2	3
23,24-Dinor-chol-5-ene-3 β ,21-diol Acetylation	23,24-Dinor-chol-5-ene- 3 β ,21-diol diacetate	<i>B</i> (1), <i>E</i> (4), <i>C</i> (5) <i>B</i> (2), <i>A</i> (3), <i>D</i> (6)	Homogenate	Slices	Slices			
			24540	8840	8040	31.8	0.97	0.95
			24830	8030	8260			
Acetylation, added carrier steroid and recrystallization	23,24-Dinor-chol-5-ene- 3 β ,21-diol diacetate		120.2	33.2	25.6			
			123.0	33.9	26.8			
			117.0	31.8	25.0			
23,24-Dinor-chol-5-ene-3 β ,20-diol, added carrier steroid and crystallized		<i>B</i> (1), <i>E</i> (5)	116.5	82.1	96.0			
			118.5	80.7	92.1	32.3	4.48	3.15
			120.3	83.7	98.8			
Acetylation and crystallization	23,24-Dinor-chol-5-ene- 3 β ,20-diol 3-acetate	<i>B</i> (2), <i>D</i> (3), <i>B</i> (4)	114.5	89.9	101.3			
			109.5	87.4	98.9			
			115.9	93.0	104.0			
Dehydrated, added carrier steroid, crystallized	23,24-Dinor-chola-5,20- dien-3 β -ol	<i>H</i>	40.8	31.6	34.1			
			39.8	31.6	32.6			
			43.3	32.4	34.9			
Acetylation and crystallization	23,24-Dinor-chola-5,20- dien-3 β -ol acetate		37.4	35.4	33.8			
			36.7	35.0	32.2			
			37.9	35.5	35.1			

Table 2. Incubation of bovine adrenal-tissue homogenate with 23,24-dinor-[7 α -³H]chol-5-ene-3 β ,20-diol (20 μ Ci)
Incubations were carried out at 37°C for 3 h. For general instructions see the Experimental section and also the legend for Table 1.

Steroid or reaction	Reaction product	T.l.c. systems used (nos. in parentheses indicate sequence)		Specific radioactivity (d.p.m./ μ mol)		Total radioactivity in metabolite (nCi)	
		Incubation no.	Incubation no.	1	2	1	2
Pregnenolone							
Acetylation	3 β -Hydroxypregn-5-en-20-one 3-acetate	B (1), E (7)	2.99 $\times 10^5$				38.8
Acetylation, added carrier steroid, recrystal- lized	3 β -Hydroxypregn-5-en-20-one 3-acetate	B (2), D (3), J (4), A (5), G (6)	3.21 $\times 10^5$				
			232.1 224.5 241.0				
Progesterone							
		B (1), B (2), F (3), J (4), K (5), G (6), B (7)	4.19 $\times 10^4$				11.5
Reduction	20 β -Hydroxypregn-4-en-3-one	G (8)	4.63 $\times 10^4$				
Added carrier steroid and crystallized	Pregn-4-ene-3,20-dione		30.8 30.0 31.9				
17-Hydroxypregnenolone							
Acetylation, added carrier steroid, crystalliza- tion	3 β ,17-Dihydroxypregn-5-en-20-one 3-acetate	B (1) B (2), G (3), E (4), K (5), B (6)	72.7 70.8 75.3				6.9
Acetylation, reduction, periodate oxidation, reduction	Androst-5-ene-3 β ,17 β -diol 3- acetate	F	4.14 $\times 10^3$				
Hydrolysis	Androst-5-ene-3 β ,17 β -diol	F	4.06 $\times 10^3$				
Corticosterone							
Acetylation, added carrier steroid, crystalliza- tion	11 β ,21-Dihydroxypregn-4-ene- 3,20-dione 21-acetate	B (1) C (2), F (3), C (4), G (5), F (6)	325.0 316.9 331.2			198.1 194.3 204.0	16.2 10.2
Cortisol							
Acetylation, added carrier steroid, crystallized	11 β -17 α ,21-Trihydroxypregn-4-ene- 3,20-dione 21-acetate	B (1) C (2), F (3), C (4), H (5), F (6)	185.1 178.8 194.1			99.7 97.2 103.2	9.0 4.6

Results

A number of radioactive metabolites was obtained from incubation with 23,24-dinor-[7 α -³H]chol-5-en-3 β -ol (I). Two of these were identified as 23,24-dinor-chol-5-ene-3 β ,20-diol (Blickenstaff, 1960; Marker *et al.*, 1942) and 23,24-dinor-chol-5-ene-3 β ,21-diol (Dusza & Bergmann, 1960) (Table 1). There was evidence of conversion into steroid hormones, but when these compounds were purified the amount of radioactivity was low.

If 23,24-dinor-chol-5-en-3 β -ol is a precursor for steroids, it would seem reasonable, by analogy with the case of cholesterol, that these hydroxylated metabolites would be intermediates in this conversion. To test this, 23,24-dinor-[7 α -³H]chol-5-ene-3 β ,20-diol was incubated with bovine adrenal tissue, and was converted into a number of steroid hormones (Table 2).

Discussion

The conversion of 23,24-dinor-chol-5-en-3 β -ol (I) into the 20- and 21-hydroxylated compounds, and of the latter into steroid hormones, demonstrates that the proposed pathway is possible, but does not prove its existence.

These results may be a further example of the lack of substrate specificity exhibited by the enzyme complex responsible for the cleavage of the cholesterol side chain (Tchen, 1968).

- Birch, A. J. & Walker, K. A. M. (1966) *J. Chem. Soc. C* 1894
 Blickenstaff, R. T. (1960) *J. Amer. Chem. Soc.* **82**, 3673
 Bush, I. E. (1961) *The Chromatography of Steroids*, pp. 358–371, Pergamon Press, London
 Chaudhuri, N. K. & Gut, M. (1969) *Methods Enzymol.* **15**, 305
 DeLuca, H. F. & Cohen, P. P. (1964) in *Manometric Techniques* (Umbreit, W. W., Burris, R. H. & Stauffer, J. F., eds.), 4th. edn., p. 132, Burgess Publishing Co., Minneapolis
 Dusza, J. P. & Bergmann, W. (1960) *J. Org. Chem.* **25**, 79
 Hall, P. F. (1964) *Endocrinology* **74**, 201
 Heard, R. D. H., Bligh, E. G., Cann, M. C., Jellinck, P. H., O'Donnell, V. J., Rao, B. G. & Webb, J. L. (1956) *Recent Progr. Horm. Res.* **12**, 45
 Marker, R. E., Crooks, H. M., Jones, E. M. & Shabica, A. C. (1942) *J. Amer. Chem. Soc.* **64**, 1276
 Shimizu, K. & Gut, M. (1965) *Steroids* **6**, 301
 Sondheimer, F. & Mechoulam, R. (1957) *J. Amer. Chem. Soc.* **79**, 5029
 Tchen, T. T. (1968) in *Functions of the Adrenal Cortex* (McKearn, K. W., ed.), vol. 1, p. 3, North-Holland, Amsterdam