1. Augdahl E. and Hassel O. *Acta Chem. Scand.* **9** (1955) 172.
2. Nesmeyanov A. and Lutsenko I. *Bull. acad. sci. U.S.S.R. Classe sci. chim.* **1943** 296—304; *Chem. Abstracts* **38** (1944) 5499₁.
3. I. G. Farbenindustrie D.R.P. 613.261 *Chem. Abstracts* **29** (1935) 5464₂.
4. Summberbell, R. and Stephens, J. J. *Am. Chem. Soc.* **76** (1954) 6404.
5. Hassel, O. and Hvostlef, J. *Acta Chem. Scand.* **8** (1954) 1953.

Received August 30, 1955.

Analysis of Steroids in Dog Adrenal Vein Blood by Counter-current Distribution

HANS CARSTENSEN

Institute of Physiology, University of Upsala, Upsala, Sweden

Besides Δ^4 -pregnene-11 β , 17 α , 21-triol-3,20-dione (cortisol) and Δ^4 -pregnene-11 β , 21-diol-3,20-dione (corticosterone) three new, very nonpolar ketonic steroids have been demonstrated in appreciable amounts in adrenal vein blood of dogs, two of them being Δ^4 -3-keto-steroids and one of these having at least one hydroxyl group.

The separation of pure corticosteroids by countercurrent distribution has been repor-

ted elsewhere¹. Adrenal vein blood was collected from anaesthetized dogs held at constant blood pressure. The blood was cooled during the collection and thereafter frozen to -20°C . After rapid thawing it was diluted with distilled water (1:1) and extracted with ethyl acetate by carrying out a fine droplet distribution of the blood into the extracting medium. The dried extract was distributed twice between *n*-hexane and 50 % ethanol in water. The combined lower phase is expected to contain all conventional corticosteroids possibly present, since their partition coefficients are less than 0.09 in this system. Different components with specific extinction at 240 $m\mu$ were separated by countercurrent distribution using 24 transfers and a sequence of different solvent systems (Table 1). The upper phase of the original distribution was subjected to a micro-Girard separation². Countercurrent distribution of the ketonic fraction (Table 2) revealed the presence of two compounds with extinction maximum at 240 $m\mu$ (designed x and z). The x-component was acylable as the partition coefficient shifted from 0.67 to 6.7 in the same system after treatment with acetyl chloride (Table 2). The blue tetrazolium³, Porter-Silber⁴ and Zimmermann⁵ reactions were negative. The last reaction was negative after oxidation with sodium bismuthate⁶ as well. A third component (designed y) appeared having an extinction maximum between 276 and 280 $m\mu$. The Kober⁷, blue tetrazolium and Porter-Silber reactions were negative, but a sharp extinction peak appeared at 260–265 $m\mu$ with the phenylhydrazine-sulfuric acid reagent. This was

Table 1.

No.	Countercurrent distribution (CC)		Partition coefficients (K)	
	Solvent system	Followed by CC No.	of polar components in adrenal vein blood	
1:	Benzene/H ₂ O	2	K	Steroid identified
		3	0.35	
2:	20 % Ethanol + 80 % H ₂ O		11.5	Cortisol
	50 % CHCl ₃ + 50 % <i>n</i> -hexane		2.57	
3:	60 % <i>n</i> -Hexane + 40 % benzene	4	0.70	Corticosterone
	5 % Ethanol + 95 % H ₂ O	5	11.5	
4:	20 % Ethanol + 80 % H ₂ O		1.6	Not identified
	CCl ₄		1.8	
5:	<i>n</i> -Hexane / H ₂ O			Only a small amount

Table 2.

Solvent system	Partition coefficients (K)						
	Pure compounds designed below			Components in adrenal vein blood			
	DC	P	Δ^4 -A	x	x-Ac	y	z
n-Hexane / 50 % ethanol + 50 % H ₂ O	0.09	0.9		10			
n-Hexane / 75 % ethanol + 25 % H ₂ O			0.08	0.67	6.7		
n-Hexane / 90 % ethanol + 10 % H ₂ O				0.41		2.70	8.1

DC: Δ^4 -Pregnene-21-ol-3,20-dione (Cortexone)

P: Δ^4 -Pregnene-3,20-dione (Progesterone)

Δ^4 -A: Δ^4 -Androstene-3,17-dione

x-Ac: The acetate of the unknown component "x"

not due to the sulfuric acid alone which in itself gave rise to a double peak at 320 and 345 μ .

1. Carstensen, H. *Acta Chem. Scand.* **9** (1955) 1026.
2. Pincus, G. and Pearlman, W. H. *Endocrinology* **29** (1941) 413.
3. Mader, W. J. and Buck, R. R. *Anal. Chem.* **24** (1952) 666.
4. Wilson, H. and Fairbanks, R. *Arch. Biochem. and Biophys.* **53** (1954) 71.
5. Wilson, H. *Arch. Biochem. and Biophys.* **52** (1954) 217.
6. Norymberski, J. K. *Nature* **170** (1952) 1074.
7. Kober, S. *Biochem. Z.* **239** (1931) 209.

Received September 6, 1955.

Semimicro Synthesis of Dihydrouracil and Uracil Labeled in Position 4, 5 or 6 with Carbon¹⁴C

PER FRITZSON*

Norsk Hydro's Institute for Cancer Research,
The Norwegian Radium Hospital,
Oslo, Norway

Recent evidence indicates the dihydropyrimidines as intermediates in the catabolism of the pyrimidine bases. Lie-

* Fellow of the "Norwegian Cancer Society", Oslo, Norway.

berman *et al.*^{1,2} have reported the isolation of an enzyme system from an anaerobic soil bacterium which reduced orotic acid to dihydroorotic acid. Di Carlo *et al.*³ suggested dihydrouracil as an intermediate in uracil catabolism from studies on the assimilation of nitrogen by *Torula utilis*. The breakdown of dihydrouracil to β -alanine in rat liver slices is shown by Fink *et al.*⁴, and Funk *et al.*⁵ reported the isolation of dihydrouracil from beef spleen.

For further investigation of the metabolism of uracil and dihydrouracil and their biological relation, a semimicro synthesis of the two pyrimidines labeled in position 4, 5 or 6 has been worked out, using potassium cyanide-¹⁴C or chloroacetic acid labeled with ¹⁴C in the 2 or 1-position, respectively, as starting materials.

126 mg of potassium cyanide containing 2 mC of ¹⁴C were treated with 244 mg of the potassium salt of chloroacetic acid, and the resultant potassium cyanoacetate was catalytically hydrogenated under a pressure of 2 atm. at 18° for 11 hours to β -alanine^{6,7}. After two crystallizations from alcohol-water the crude β -alanine was reacted with 160 mg of potassium cyanate at 25°, forming the potassium salt of β -ureidopropionic acid^{8,9}. Treatment of the crude product with hydrochloric acid and subsequent heating of the dry substance to 170° yielded 119 mg (56 % based on chloroacetic acid) of dihydrouracil-4-¹⁴C crystallized from water. On recrystallization from water, adding charcoal, the product melted at 274—275° (reported¹⁰ m. p. 275—276°). The theoretical specific activity was 9.1 μ C per mg.