Identification of 3α,17α,21-Trihydroxy-5α-pregnane (Allotetrahydro-17α-hydroxy-cortexone) and 3α,21-Dihydroxy-5α-pregnane (Allo-tetrahydro-cortexone) in Human Urine

During the past few years, several corticosteroids with an α-ketolic side chain and a 5α-pregnane configuration, have been identified in human urine. These are: 3α, 11β, 17α, 21-tetrahydroxy-5α-pregnane 20-one (allo-tetrahydrocortisol)[1,2]; 3α, 17α, 21-trihydroxy-5α-pregnane 11, 20-dione (allo-tetrahydro-cortisone)[1,3]; 3α, 11β, 21-trihydroxy-5α-pregnane 20-one (allo-tetrahydrocorticosterone)[4,5]); and 3α, 21-dihydroxy-5α-pregnane 3, 20-dione (allo-tetrahydro-11-dehydrocorticosterone)[6].

In this paper we report the isolation and identification, in human urine, of two new corticosteroids with an α-ketolic side chain and a 5α-pregnane structure.

500 mg of Metopirone (2-methyl-1, 2-bis(3-pyridyl-1-propanone) were administered to three normal men every 4 h for 24 h and the urines were collected during the 24 h after the last dose. The urines were hydrolysed with enzymes of the succus entericus of *Helix pomatia* and extracted with dichloromethane (3 × 0.5 vol.). The dichloromethane solution was washed with Na_2CO_3 solution (9% w/v) and subsequently with water.

The material extracted with dichloromethane was chromatographed in the chloroform/formamide system. Three zones were cut from the chromatogram: zone I from the origin to 15 cm; zone II from 15 cm to 25 cm; zone III from 28 cm to the solvent front.

Identification of 3α, 17α, 21-trihydroxy-5α-pregnane 20-one (allo-tetrahydro-17α-hydroxy-cortexone; allo-THS). Zone II of the chloroform/formamide chromatogram was eluted and chromatographed successively in the di-isopropyl-ether/formamide-water (10/1-1 by vol.), toluene/propanediol, and iso-octane-toluene/methanol-water (4-1/5-5 by vol.) systems. In the di-isopropyl-ether/formamide-watersystem, a spot reducing tetrazolium blue[7] and having an R_{THS}[8] = 1.48 was detected. After isolation, this substance had the same migration as that of synthetic 3α, 17α, 21-trihydroxy-5α-pregnane 20-one[9] in the four systems indicated above. The acetate of the isolated compound had the same migration as that of synthetic tetrahydro 17α-hydroxy-cortexone 3, 21-diacetate in the ligroin/propanediol system.

The product of sodium bismuthate oxidation[10] gave a positive Zimmermann reaction[11] and had the same Rf value as androsterone (3α-hydroxy-5α-androstan-17-one), both in the ligroin/propanediol system and in the hexane-benzene (1/1 by vol.)/propanediol one. The acetate of the oxidation product had the same Rf value as androsterone acetate on paper chromatograms, with iso-octane as the mobile phase but without stationary phase[12].

Identification of 3α, 21-dihydroxy-5α-pregnane 20-one (allo-tetrahydro-cortexone; allo-THDOC). Zone III of the chloroform/formamide chromatogram was eluted and chromatographed successively in the ligroin/propanediol, iso-octane/propanediol, and iso-octane/methanol-water (5/3-2 by vol.) systems. In the iso-octane/propanediol system, a spot having an R_{THDOC}[13] = 1.65 was detected. After elution and chromatography in the systems indicated above, only one spot, giving a positive reaction to tetrazolium blue and having the same migration as that of synthetic allo-tetrahydro-cortexone (allo-THDOC), was found. This substance did not give a positive Porter-Silber test[14].

The Table gives the Rf values for the isolated steroid, synthetic THDOC isomers and, 21-hydroxy-pregnenolone in the three chromatographic systems.

With sodium bismuthate oxidation, the steroid obtained was converted to a more polar substance by the formation of a carboxylic function at C-20 (aetio acid). The Rf value of this product, relative to tetrahydro-cortexone, was 0.35 in the benzene/formamide system. The acetate of the isolated compound had the same migration as that of synthetic allo-tetrahydro-cortexone 3, 21-diacetate, using paper chromatography without stationary phase and iso-octane as solvent. The Rf value of this acetate, compared with that of its axial isomer (3β, 21-dihydroxy 5β-pregnane 20-one 3, 21-diacetate) was 1.81. In previous work with these chromatographic systems, we

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Rf values for the isolated compound (Zone III of the chloroform/formamide system) and tetrahydro-cortexone isomers

Steroids	Chromatographic systems		
	Ligroin/propanediol	Iso-octane/propanediol	Iso-octane/methanol-water (5/3-2) v/v
	R_{THDOC} (40 h)	R_{THDOC} (48 h)	R_{THDOC} (15 h)
Isolated compound of Zone III	1.85	1.65	1.60
3α,21-Dihydroxy-5β-pregnan-20-one (THDOC)	1	1	1
3α,21-Dihydroxy-5α-pregnan-20-one (allo-THDOC)	1.84	1.65	1.61
3β,21-Dihydroxy-5α-pregnan-20-one	0.92	0.86	1.16
3β,21-Dihydroxy-5β-pregnan-20-one (3β-THDOC)	1.66	1.52	1.95
3β,21-Dihydroxy-5-pregnen-20-one	0.73	0.60	0.88

observed that, in two steroid series, the acetate of 3β, 5β-axial configuration was more polar than that of its 3α, 5α-axial isomer.

We used micro-tetrazolium blue reaction for quantitative values, and obtained 200–250 μg/24 h for allo-tetrahydro 17α-hydroxy-cortexone and 100–150 μg/24 h for allo-tetrahydro-cortexone, in the subjects studied.

Discussion. The hormone of the adrenal cortex, 17α, 21-dihydroxy-pregn-4-en 3, 20-dione (Compound S), is metabolized into different hydrogenated derivatives which have been isolated from human urine. These are: 3α-17α, 21-trihydroxy-5β-pregnane 20-one (tetrahydro-17α-hydroxy-cortexone-THS)[15]; 3α, 17α, 20ξ, 21-tetrahydroxy-5β-pregnane (hexahydro-17α-hydroxy-cortexone)[16]; 17α, 21-di-hydroxy-5β-pregnane 3, 20-dione (dihydro-17α-hydroxy-cortexone)[17]; and 6β, 17α, 21-trihydroxy-pregn-4-en 3, 20-dione (6β, 17α-dihydroxy-cortexone)[18]. The allo-tetrahydro-17α-hydroxy-cortexone identified in this work, is a new metabolite of 17α-hydroxy-cortexone. It is to be pointed out that 17α, 21-dihydroxy-pregnenolone (3β, 17α, 21-trihydroxy-pregn-5-en 20-one) can also be converted *in vivo* into allo-tetrahydro-17α-hydroxy-cortexone, as we have recently shown[19].

The hormone cortexone, 21-hydroxy-pregn-4-en 3, 20-dione, is metabolized into 3α, 21-dihydroxy-5β-pregnane 20-one (tetrahydroxy-cortexone)[16] and 21-hydroxy-5β-pregnane 3, 20-dione (dihydrocortexone)[17]. The 3α, 21-dihydroxy-5α-pregnane 20-one (allo-tetrahydro-cortexone), identified in this work, is a new metabolite of this hormone.

Résumé. Le 3α, 17α, 21-trihydroxy 5α-pregnane (allo-tétrahydro-17α-hydroxy-cortexone) et le 3α, 21-dihydroxy 5α-prégnane (allo-tétrahydro-cortexone) ont été identifiés dans l'urine humaine. Ces composés avaient les mêmes migrations chromatographiques avant ou après acétylation ou oxydation que les respectifs stéroïdes de synthèse.

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Release by Sympathetic Stimulation of α-Methylnoradrenaline Stored in the Heart after Administration of α-Methyldopa

α-Methyldopa causes a long-lasting depletion of heart and brain noradrenaline[1–3]. CARLSSON and LINDQVIST found that α-methyldopamine and α-methylnoradrenaline accumulated in the brain after administration of α-methyldopa. They expressed the view that the decarboxylation products of α-methyldopa may possibly take over the functions of the physiological amines. This hypothesis has gained some support from the observation that transmission over sympathetic pathways is not inhibited in dogs whose noradrenaline stores have been depleted by 56% following α-methyldopa[4]. On the other hand, this loss of noradrenaline may have been insufficient to cause functional failure.

It was demonstrated recently that after administration of α-methyldopa the noradrenaline of the heart is partially replaced by an equipressor amount of α-methylnoradrenaline[5]. In order to find out whether sympathetic stimulation releases α-methylnoradrenaline concomitantly with noradrenaline, we have done the following experiments.

Rabbits were given four intravenous doses of 100 mg/kg of dl-α-methyldopa in the course of two days. 16 h after the last injection, the heart with the sympathetic nervous supply was isolated and perfused[6]. Untreated animals served as controls. The perfusion fluid was collected before, during and after sympathetic stimulation or during an infusion of dimethylphenyl-piperazinium iodide (DMPP). The catecholamines were adsorbed on alumina. After termination of the perfusion experiment, the right ventricle was homogenized in trichloroacetic acid and the catecholamines were adsorbed on alumina[7]. The pressor activity of the alumina eluates was assayed against standard doses of l-noradrenaline and l-α-methylnoradrenaline on the pithed rat. In this test preparation, the ratio of equipressor doses of l-α-methylnoradrenaline/l-noradrenaline (expressed as bases) was 0.95 (0.86–1.03). In aliquots of the alumina eluates, the concentrations of noradrenaline and adrenaline were estimated by differential fluorometry[8]. α-Methylnoradrenaline exhibited only 3.8% of the fluorescence of noradrenaline and less than 1% of that of adrenaline. Differential estimation of noradrenaline and α-methylnoradrenaline could therefore be performed by using the biological and fluorometric assay procedures. In control experiments, when there was no α-methylnoradrenaline in heart extracts or perfusates obtained after sympathetic stimulation or DMPP, the concentration of noradrenaline found biologically agreed well with the result of the chemical estimation (Figure). If, however, the myocardium or the perfusates were obtained from rabbits pretreated with α-methyldopa, the % ratio, fluorometric activity/pressor activity, fell significantly to less than 50% (Figure).

The noradrenaline concentration in the right ventricle of the perfused hearts of untreated rabbits was 1.39 ± 0.12 μg/g as estimated on the blood pressure, and 1.40 ± 0.09

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