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Short Communication

SYNTHESIS OF TETRAHYDROCORTISOL 3-GLUCURONIDE

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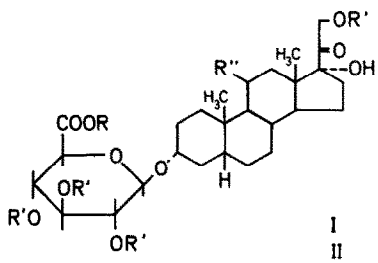
SUMMARY

The chemical synthesis of tetrahydrocortisol 3-glucuronide (11β, 17, 21-trihydroxy-20-oxo-5β-pregnan-3α-yl-β-D-glucopyranoside) uronic acid (I) and its 21-acetoxy triacetate methylester derivative [methyl-(21-acetoxy-11β, 17-dihydroxy-20-oxo-5β-pregnan-3α-yl-2′, 3′, 4′-tri-O-acetyl-β-D-glucopyranoside) uronate (II)] is described.

TETRAHYDROCORTISOL glucuronides have been found and determined in human fluids by several authors [1–3]. Similarly, when cortisone [4] or tetrahydrocortisol [5] were incubated with uridine diphosphate glucuronic acid (UDPGA) and liver microsomes, the formation of tetrahydrocortisol glucuronides was observed. Foggitt and Kellie [6] isolated I from urine; after methylation and acetylation, the authors obtained II and identified it by microchemical methods. So far, only chromatographical properties of I and II are known. The 3-glucuronide of tetrahydrocortisol has now been chemically synthesized, in order to unequivocally characterise the compound and to use it for further studies on the biogenesis and metabolism.



	R	R'	R''
I	-H	-H	-OH
II	$-CH_3$	-Ac	-OH
III	$-CH_3$	-Ac	=O

EXPERIMENTAL

0·06 ml of acetic anhydride and 0·55 ml of absolute benzene were added to a solution of 190 mg of tetrahydrocortisol in 0·3 ml of dry pyridine. The mixture was allowed to stand at room temperature overnight. After evaporation of the solvents, the residue of the partially acetylated steroid was purified by column chromatography on SiO_2 with chloroform–methanol (95:5, v/v). Crystallisation from ether–hexane yielded 110 mg of tetrahydrocortisol 21-acetate (3α, 11β, 17-trihydroxy-5β-pregnan-21-yl-acetate).*

*Tetrahydrocortisol 21-acetate is now offered by Mann Research Laboratories, Inc., New York, but was not available at the time of this investigation.

A mixture of 100 mg of tetrahydrocortisol 21-acetate, 450 mg of methyl-(2, 3, 4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyran) uronate[7] and 280 mg of freshly prepared silver oxide[8] in 10 ml anhydrous benzene was stirred in the dark for 48 h at room temperature according to the procedure of Schneider[9]. The silver salts were filtered out, and the filtrate was evaporated yielding an oil. The oil was dissolved in 3 ml of acetone and poured into 20 ml of water[10]. The derivatives of the steroid precipitated and were filtered out through a pad of celite. They were then dissolved in the filter with CH_2Cl_2. After drying over Na_2SO_4, the solution was concentrated to 2–3 ml and chromatographed on a SiO_2 column (40 g) with ether-petroleum ether (50–70°)(85:15, v/v). By collecting the eluate in 10 ml fractions, the desired product was found in fractions 24–34. The evaporated eluate was crystallised from 2·5 ml of benzene and 5 ml of petroleum ether, yielding 55 mg of long needles of II. The analytical sample was recrystallised from a small volume of chloroform-ether. Mp 191–194°; $[\alpha]_D^{20} + 29°$ ($CHCl_3$; c 0·45). Mass spectrum, m/e 724 (3%; M^+); 664 (2%); 636 (1%); 433 (1%); 391 (3%); 390 (5%); 373 (18%); 331 (9%); 330 (12%); 317 (100%); 257 (63%); 155 (73%); 43 (71%); a majority of the abundant fragments in the mass spectrum could be interpreted as arising from loss of MeO, COOMe, and acetic acid fragments, and by cleavage of the glycosidic bond.

Anal. Calcd for $C_{36}H_{52}O_{15}$: C, 59·7% H, 7·23%.
Found: C, 59·5% H, 7·28%.

II gave a positive blue tetrazolium reaction[11].

5 mg of II was oxydized by pyridine-chromium oxide. Four mg of methyl-(21-acetoxy-17-hydroxy-11β, 20-dioxo-5β-pregnan-3α-yl-2′, 3′, 4-tri-O-acetyl-β-D-glucopyranoside) uronate (III) was crystallised from 2 drops of CH_2Cl_2 and 10 drops of ether. Mp 209–214°; $[\alpha]_D^{20} + 37°$ ($CHCl_3$; c 0·1) (lit.[12]: 209–212°; $[\alpha]_D$ + 37·8°).

Mass spectrum, m/e 722 (1%; M^+); 622 (1%); 634 (1%); 431 (2%); 389 (35%); 388 (11%); 371 (18%); 329 (42%); 328 (29%); 317 (97%); 257 (69%); 155 (94%); 43 (100%).

For saponification of the protecting groups, 20 mg of II was stirred in 1·8 ml of 0·2 N methanolic sodium methylate for 15 min. One ml of water was added and 10 min later 1 ml of 1 N acetic acid. After evaporation, the residue was dissolved in 3 ml of water and the pH brought to 2 (glass electrode) by addition of 1 N hydrochloric acid. The solution was poured onto an Amberlite XAD-2 column [13]. When all solvent had passed, the column was washed with 12 ml of water. Subsequently, the column was eluted with 10 ml of ethanol and the ethanolic solution taken to dryness. Crystallisation from methanol–ethyl acetate gave 10 mg of I. Mp 192–198° (dec.); $[\alpha]_D^{20} + 16·6°$ (MeOH; c 0·25).

Anal. Calcd for $C_{27}H_{42}O_{11} \cdot H_2O$: C, 58·5%; H, 8·01%.
Found: C, 58·8%; H, 8·06%.

The compound was shown to be homogenous by paper chromatography in the systems:

a. n-butyl acetate: toluene: n-butanol: methanol: water: acetic acid (50:40:10:50:45:5 by vol)

b. n-butyl acetate: toluene: n-butanol: water: acetic acid (50:10:40:9:1 by vol).

I gave a strong positive tetrazolium reaction and positive Tollens reaction [14]. By enzymatic hydrolysis of I with β-glucuronidase, the corresponding free steroid was formed. It was identical with an authentic sample of tetrahydrocortisol.

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