obtained, mp 125—126°. *Anal.* Calcd. for $C_5H_4O_6N_4$(VI): C, 27.79; H, 1.87; N, 25.93. Found: C, 27.44; H, 1.85; N, 25.54. Compound VII was obtained also by reaction of VI with MeOH–NaOMe solution: To a suspension of 1 g of VI in 30 ml of dry MeOH, 5 ml of MeOH–NaOMe solution was added with cooling. The mixture was allowed stand at room temperature for 3.5 hours (crystals separated). The reaction mixture was treated same to above method, yield 0.38 g(42%), mp 155—156°. This compound was identified with an authentic specimen of VII.

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Studies on Steroid Conjugates. VI. Synthesis of 17α-Estradiol 17-N-Acetylglucosaminide[1]

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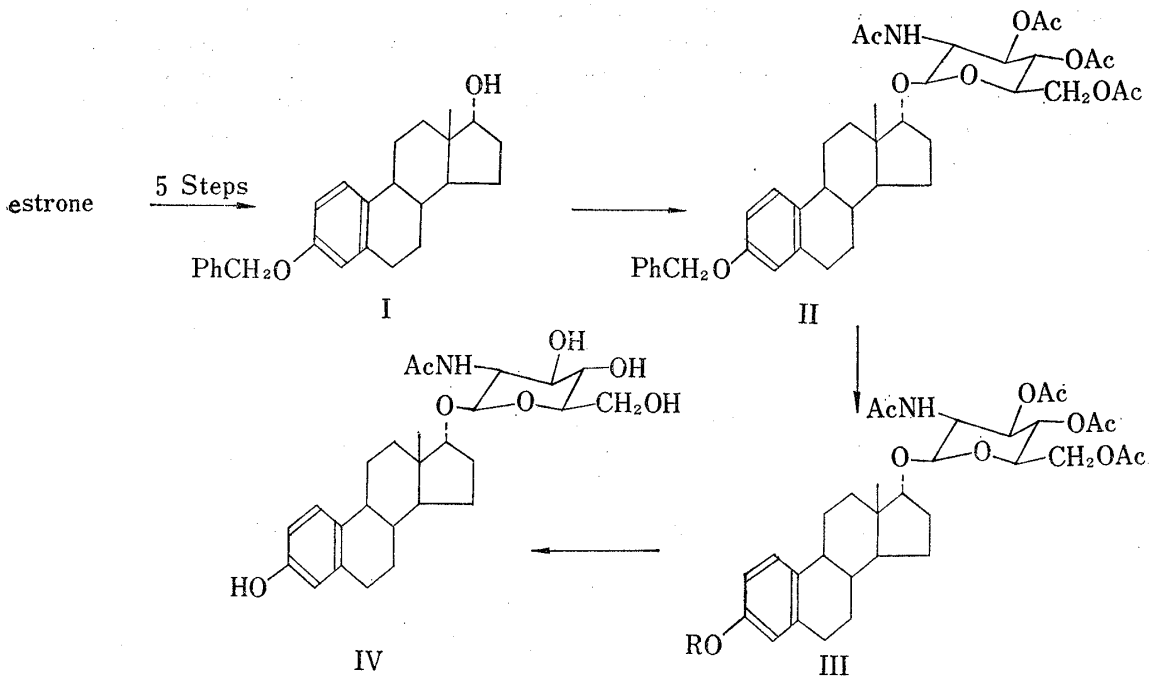
In 1964 Layne, *et al.* isolated first 17α-estradiol 17-N-acetylglucosaminide (IV) from the rabbit urine treated with β-glucuronidase after a large dosage of estrone benzoate.[3] As a series of our studies on the modified steroids the metabolic fate of 3-deoxyestrone in the rabbit has previously been investigated. In our experiment, however, it was found that 17α-estradiol, one of the principal metabolites, was excreted solely in the form of the 17-glucuronide, and no evidence was obtained for *in vivo* formation of steroidal N-acetylglucosaminide.[4] It was thereby assumed that the observed difference would be ascribable to the breed. In order to clarify the possible conjugation of N-acetylglucosamine with the estrogens in the domestic rabbit and the behaviors of the conjugate against the enzymatic hydrolysis, the availability of the authentic sample has become requisite. In this paper we wish to report the synthesis of the titled compound from estrone by an unequivocal route (see Chart 1).

For this purpose 17α-estradiol 3-benzyl ether (I), derivable from estrone in five steps,[5] was employed as a starting material. The Koenigs–Knorr reaction has already been used with success for the preparation of the steroidal N-acetylglucosaminides.[6] Consequently an introduction of the sugar moiety was attempted by the use of this reaction. When I and acetochloroglucosamine (1α-chloro-2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-D-glucopyranose) were stirred in dry toluene with cadmium carbonate as the condensing agent, the reaction proceeded with ease to give 3-benzyloxyestra-1,3,5(10)-trien-17α-yl-2′-acetamido-2′-deoxy-3′,-4′,6′-tri-O-acetyl-β-D-glucopyranoside (II) almost quantitatively. The formation of the β-glucoside linkage was verified by the inspection of the nuclear magnetic resonance (NMR) spectrum. The anomeric proton of the sugar moiety in II appeared at 4.62 ppm as doublet (J=7.8 cps) indicating a *trans*-diaxial relationship to the vicinal 2′-proton. The catalytic

1) This paper constitutes Part IL of the series entitled "Analytical Chemical Studies on Steroids"; Part XLVIII: T. Nambara, M. Nokubo, and Y.H. Bae, *Chem. Pharm. Bull.* (Tokyo), **19**, 2096 (1971).
2) Location: *Aobayama, Sendai.*
3) D.S. Layne, N.A. Sheth, and R.Y. Kirdani, *J. Biol. Chem.*, **239**, 3221 (1964).
4) T. Nambara and M. Numazawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 990 (1971).
5) T. Nambara, M. Numazawa, and H. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 1725 (1969).
6) R. Hirschmann, R.G. Strachan, P. Buchschacher, L.H. Sarett, S.L. Steelman, and R. Silber, *J. Am. Chem. Soc.*, **86**, 3903 (1964); G. Sauer, M. Matsui, R. Bloch, J.S. Liang, and D.K. Fukushima, *J. Org. Chem.*, **34**, 3525 (1969).



a : R=H, b : R=Ac, Ph=phenyl

Chart 1

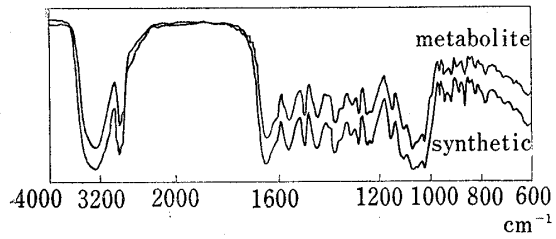


Fig. 1. Infrared Spectra of 17α-Estradiol 17-N-Acetylglucosaminide (KBr Disk)

hydrogenation over palladium–on–charcoal afforded 3-hydroxyestra-1,3,5(10)-trien-17α-yl-2′-acetamido-2′-deoxy-3′,4′,6′-tri-O-acetyl-β-D-glucopyranoside (IIIa) with loss of the benzyl group at C-3. Usual acetylation with acetic anhydride and pyridine gave the 3-acetate (IIIb). The removal of the protecting groups in the sugar moiety was then achieved by treatment with methanolic potassium hydroxide. Thus the desired 17α-estradiol 17-N-acetylglucosaminide (IV) was furnished as colorless needles.

Upon direct comparison with the metabolite isolated from the rabbit urine[3] the synthetic specimen proved to be entirely identical in every respect. As was shown in Fig. 1 the infrared (IR) spectra of two samples were completely superimposable.

Experimental[7]

3-Benzyloxyestra-1,3,5(10)-trien-17α-yl-2′-acetamido-2′-deoxy-3′,4′,6′-tri-O-acetyl-β-D-glucopyranoside (II)——To a solution of 3-benzyloxyestra-1,3,5(10)-trien-17α-ol (I)[5](100 mg) in anhydrous toluene (5 ml) were added acetochloroglucosamine[8](600 mg) and $CdCO_3$ (300 mg) in several portions for 3 days, and the suspended solution was stirred at 53—57°. The reaction mixture was diluted with AcOEt and the precipitate was filtered off. The filtrate was concentrated *in vacuo* to give an oily residue. The crude product was submitted to the preparative thin–layer chromatography (TLC) on silica gel HF using ether as developing

7) All melting points were taken on a micro hot–stage apparatus and are uncorrected. IR spectral measurements were run on JASCO Model IR-S spectrometer. NMR spectra were recorded on Hitachi Model R-20 spectrometer using tetramethylsilane as an internal standard (TMS=0.0 ppm). Abbreviation used n=narrow, s=signlet, d=doublet, and m=multiplet.

8) D.H. Leaback and P.G. Walker, *J. Chem. Soc.*, **1957**, 4574.

solvent. Recrystallization from EtOH gave II (190 mg) as colorless leaflets. mp 210—212°. $[\alpha]_D^{18}+3.6°$ ($c=0.14$, $CHCl_3$). *Anal.* Calcd. for $C_{39}H_{49}O_{10}N$: C, 67.71; H, 7.14; N, 2.02. Found: C, 67.49; H, 7.42; N, 2.24. NMR (5% solution in $CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 3.72 (1H, d, $J=5$ cps, 17β-H), 4.12 (2H, nm, $W_{\frac{1}{2}}=7.2$ cps, 6'-H), 4.62 (1H, d, $J=7.8$ cps, 1'-H), 4.95 (2H, s, $C_6H_5CH_2$-), 7.27 (5H, s, $C_6H_5CH_2$-).

3-Hydroxyestra-1,3,5(10)-trien-17α-yl-2'-acetamido-2'-deoxy-3',4',6'-tri-O-acetyl-β-D-glucopyranoside (IIIa)——A solution of II (163 mg) in AcOEt (10 ml) was shaken with 5% Pd/C (100 mg) overnight under a stream of H_2 gas at room temperature. After removal of the catalyst by filtration the filtrate was concentrated *in vacuo* to give a crystalline product. Recrystallization from acetone–hexane gave IIIa (151 mg) as colorless needles. mp 245—246°. $[\alpha]_D^{20}-42.7°$ ($c=0.12$, MeOH). *Anal.* Calcd. for $C_{32}H_{43}O_{10}N$: C, 63.88; H, 7.20; N, 2.33. Found: C, 63.91; H, 7.14; N, 2.49. NMR (5% solution in $CDCl_3$) δ: 0.67 (3H, s, 18-CH_3), 4.13 (2H, nm, $W_{\frac{1}{2}}=7$ cps, 6'-H), 4.47 (1H, d, $J=8$ cps, 1'-H).

3-Acetoxyestra-1,3,5(10)-trien-17α-yl-2'-acetaimdo-2'-deoxy-3',4',6'-tri-O-acetyl-β-D-glucopyranoside (IIIb)——IIIa (25 mg) was treated with pyridine and Ac_2O at room temperature overnight. Usual work-up followed by recrystallization from acetone–hexane gave IIIb (27 mg) as colorless needles. mp 145—147°. $[\alpha]_D^{19}-13.6°$ ($c=0.11$, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{45}O_{11}N$: C, 63.44; H, 7.05; N, 2.17. Found: C, 63.35; H, 7.08; N, 2.23. NMR (5% solution in $CDCl_3$) δ: 0.70 (3H, s, 18-CH_3), 3.73 (1H, d, $J=5$ cps, 17β-H), 4.17 (2H, nm, $W_{\frac{1}{2}}=7$ cps, 6'-H), 4.66 (1H, d, $J=7.5$ cps, 1'-H).

3-Hydroxyestra-1,3,5(10)-trien-17α-yl-2'-acetamido-2'-deoxy-β-D-glucopyranoside (IV)——To a solution of IIIa (57 mg) in MeOH (3 ml) was added 1N methanolic KOH (0.35 ml) and allowed to stand at room temperature overnight. To the resulting solution was added Amberlite IR-120 H-form (*ca.* 1 ml) and stirred for 10 min. The reaction mixture was filtered and washed with MeOH. The filtrate and washings were combined and concentrated *in vacuo*. Recrystallization of the crude product from aq. MeOH gave IV (40 mg) as colorless needles. mp 170—177°. $[\alpha]_D^{19}-20.0°$ ($c=0.10$, MeOH). *Anal.* Calcd. for $C_{26}H_{37}O_7N\cdot\frac{1}{2}H_2O$: C, 64.44; H, 7.90; N, 2.89. Found: C, 64.51; H, 8.27; N, 2.98. TLC (Silica gel G) *Rf*: 0.35 (AcOEt/MeOH (5:1)), 0.61 ($CHCl_3$/EtOH (7:3)). IR spectra and TLC comparison with the metabolite isolated from the rabbit urine[3] showed the identity of two samples.

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Studies on Peptides. XXXIII.[1,2] N^{ε}-β,β,β-Trichloroethyloxycarbonyllysine

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The β,β,β-trichloroethyloxycarbonyl (TrOC) group was first introduced by Woodward, *et al.*[4] as an amino protecting group in the synthesis of cephalosporin C. Later Windholz and Johnston[5] studied its applicability in organic synthesis. This masking group is resistant to the action of HCl in dioxane or trifluoroacetic acid (TFA) but can be removed under very mild conditions by treatment with Zn in acetic acid or boiling methanol for a short period.

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1) Part XXXII: H. Yajima and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **19**, 1905 (1971).
2) Amino acids, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commision of Biochemistry Nomenclature in July 1965 and July 1966; *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967). Z(OMe)=*p*-methoxybenzyloxycarbonyl, Z=benzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, OBzl=benzyl ester.
3) Location: *Sakyo-ku, Kyoto.*
4) R.B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan and H. Vorbrüggen, *J. Am. Chem. Soc.*, **88**, 852 (1966).
5) T.B. Windholz and D.B.R. Johnston, *Tetrahedron Letters*, **1967**, 2555.