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Steroids. IV.[1] Revised Structure for the Product from 3β-Acetoxycholest-5-ene with Nitrous Acid

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The compound (**4**), which was obtained from 3β-acetoxycholest-5-ene with nitrous acid and was assigned 6-acetoximino-3β-acetoxycholestan-5α-ol nitrite (**2**), is revised as 3β,5α-diacetoxy-6-nitriminocholestane on the basis of its chemical behaviors and eventually by identification with the compound prepared from 3β-acetoxy-6-hydroximinocholestan-5α-ol (**14**) *via* two steps.

Narayanan, *et al.*[3] reported that the compound, $C_{31}H_{50}O_6N_2$, obtained by treatment of 3β-acetoxycholest-5-ene (**1**) with acetic acid, sodium nitrite, and nitric acid was established as 6-acetoximino-3β-acetoxycholestan-5α-ol nitrite (**2**) on the basis of its spectral properties and was eventually identified with the nitrite (**2**) prepared from 6-acetoximino-3β-acetoxycholestan-5α-ol (**3**) with nitrosyl chloride. Subsequently, we obtained the same compound by a similar procedure to that of Narayanan, *et al.* and discussed its reaction behaviors on catalytic reduction and alumina on the basis of the structure (**2**).[1,4] Quite recently, Narayanan, *et al.*[5] have reported that the identification of the compound mentioned above was incorrectly carried out, misleading to the nitrite (**2**) and its structure was revised as 3β,5α-diacetoxy-6-nitriminocholestane (**4**) by another synthetic procedure. Under the circumstances, we re-investigated our compound in order to obtain the correct conclusion by our hands. We now report our findings leading to the same result obtained by Narayanan, *et al.*

The compound (**4**), which was obtained by treating 3β-acetoxycholest-5-ene (**1**) with sodium nitrite in the presence of acetic acid, and conc. sulfuric acid,[4] shows the same spectral properties as those of compound obtained by Narayanan, *et al.* Treatment of the compound (**4**) with methanolic potassium hydroxide solution at room temperature afforded the potassium salt (**5**) containing two nitrogen atoms which with 10% acetic acid or 10% hydrochloric acid was converted into the *sec*-nitramine (**6**). Further, the *sec*-nitramine (**6**) turned into 3β,5α-dihydroxycholestan-6-one (**7**) on refluxing with methanolic potassium hydroxide solution.[4] The *sec*-nitramine (**6**) shows bands corresponding to the NO_2 group at 1580 and 1315 cm^{-1} in the infrared (IR) spectrum (KBr) and absorption maximum at 244 mμ in the ultraviolet (UV) spectrum (EtOH), indicating the likely presence of the $HNNO_2$ group.[6] Its nuclear magnetic resonance (NMR) spectrum (100 MHz, acetone-d_6) exhibits signals due to three protons disappeared on deuterium oxide-addition and one vinyl proton at δ 2.88 and 5.88, respectively. These spectral data suggest the sec-nitramine (**6**) to be 3β,5α-dihydroxy-6-nitraminocholest-6-ene. If the compound (**4**) is the nitrite (**2**) as assigned previously, the oxime (**9**) should be obtained through the potassium salt (**8**) by hydrolysis and subsequent acidification. However, the oxime (**9**) can not be supported by the spectral data obtained

1) Part III: M. Onda and K. Takeuchi, *Chem. Pharm. Bull.* (Tokyo), **21**, 1287 (1973).
2) Location: *Minato-ku, Tokyo 108, Japan.*
3) C.R. Narayanan, M.S. Parkar, and M.S. Wadia, *Tetrahedron Letters*, **1970**, 4703.
4) M. Onda and A. Azuma, *Chem. Pharm. Bull.* (Tokyo), **19**, 859 (1971); *ibid.*, **20**, 1467 (1972).
5) C.R. Narayanan, M.S. Parkar, and P.S. Ramaswamy, *Chem. Ind.* (London), **1974**, 208.
6) J.P. Freeman, *J. Org. Chem.*, **26**, 4190 (1961).

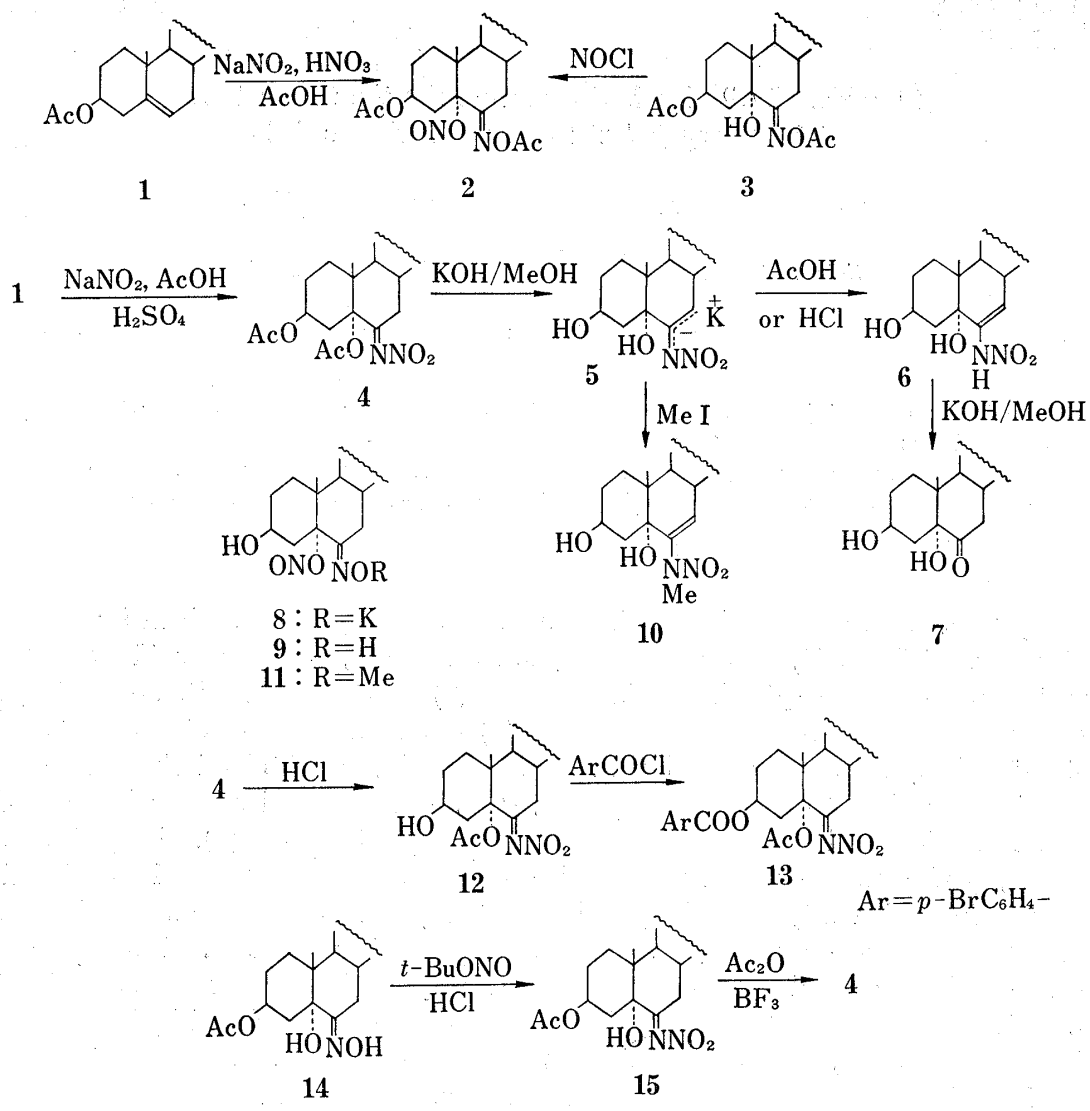

Chart 1

above. Further, the potassium salt (**5**) was treated with methyl iodide to give the *tert*-nitramine (**10**) whose spectral data (see Experimental) also show the presence of the $MeNNO_2$ group[6] and the 7-vinyl proton, precluding the oxime ether (**11**) for the structure of the *tert*-nitramine (**10**). Acidic hydrolysis of the compound (**4**) with hydrochloric acid at room temperature gave the mono-acetate (**12**), in which the 3β-OH group was confirmed on the basis of its spectral data (see Experimental). On treatment with *p*-bromobenzoyl chloride the mono-acetate (**12**) afforded the di-ester (**13**). At this stage, we can surely deny the nitrite (**2**) for the compound (**4**) obtained by us. Since the chemical and spectral properties of the compound (**4**) appears to coincide with those of 3β,5α-diacetoxy-6-nitriminocholestane, we attempted to prepare it from 3β-acetoxy-6-hydroximinocholestan-5α-ol (**14**).[7]

Treatment of the oxime (**14**) with *tert*-butyl nitrite in the presence of hydrogen chloride and subsequently with water gave the nitrimine (**15**) whose IR spectrum (KBr) showed bands at 3460 (OH), 1715 (OAc), 1630 (C=N), 1567 and 1320 cm^{-1} (NO_2). On acetylation with acetic anhydride in the presence of boron trifluoride etherate, the nitrimine (**15**) afforded 3β,5α-diacetoxy-6-nitriminocholestane which was identified with the compound (**4**) by mixed mp and comparisons of the spectral data.

7) G. Drefahl and K. Ponsold, *Chem. Ber.*, **91**, 271 (1958).

In a previous paper[4] we reported that the catalytic reduction of the compound (**4**) over Adams' platinum in acetic acid gave 3β,5α-diacetoxy-6β-aminocholestane (**16**) as a main product and its formation pathway with an acyl migration was presumed on the basis of the structure of the nitrite (**2**). Now, it is natural for the compound (**4**) to afford the amine (**16**) by reduction and such the formation pathway (*loc. cit.*) should be withdrawn. However, the fact that the catalytic reduction of the compound (**4**) in dioxane gave the ketone (**17**) and no compound containing the nitrogen atom remains to be re-examined. For formation of the 6=O group, addition of the oxygen atom in the NO_2 group to C-6 would be considered to occur at some stage. The compound (**4**) remained unchanged on treatment with Adams' platinum in the absence of hydrogen in dioxane. During the reduction about four moles of hydrogen were absorbed and ammonia was detected at the end of the reduction.

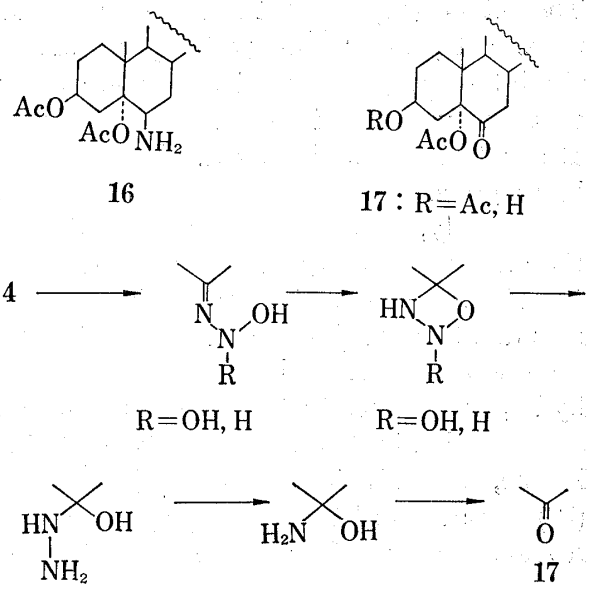

Chart 2

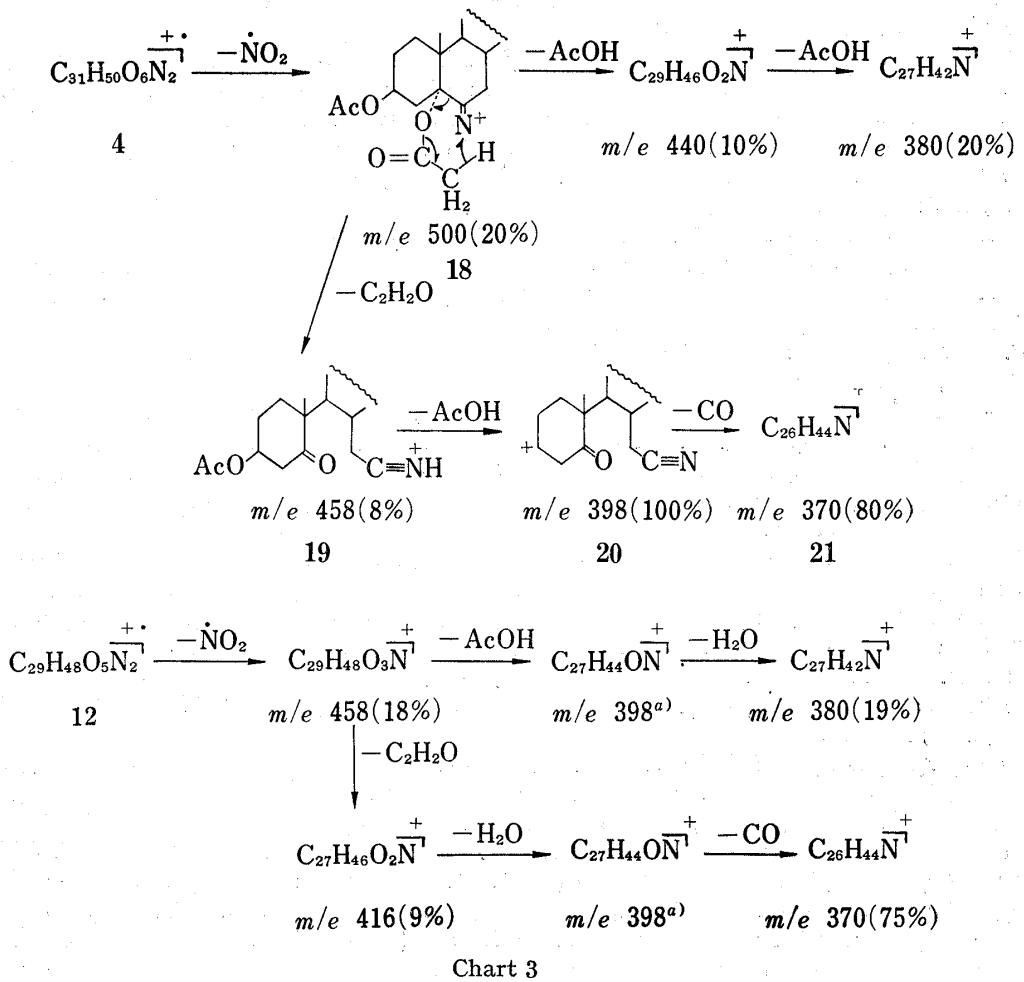

Chart 3

The figures in parentheses show the relative intensity.
a) Base peak

The ketone (**17**) was not reduced over Adams' platinum in dioxane. Taking into account these facts, a formation pathway would be deduced as depicted in Chart 2. The structure of the interesting compound from the compound (**4**) *via* alumina-induced reaction, which was tentatively assumed to possess the 5α,6α-epoxy and 6β,7β-nitrosimino groups,[1] should be also withdrawn and revised. Re-investigation and X-ray analysis of this compound are in progress.

Finally, we briefly comment on the mass spectrum (MS) of the compound (**4**) in which the molecular ion is undetectable and the ion of greatest mass observed is the $(M-NO_2)^+$ (**18**). Plausible fragmentations are deduced as follows. The ion (**18**) would give successively the ions due to the *m/e* 440 and 380 by double eliminations of acetic acid. On the other hand, there is a characteristic fragment which may be considered to result from the ion (**18**). A hydrogen transfer to the nitrogen atom and synchronous acyl fission in the 5α-OAc group accompanied by bond fission between C-5 and C-6 appear to give the ion (**19**) by the loss of ketene, from which the ion (**20**) (base peak) results *via* elimination of acetic acid. The ion (**20**) would afford the ion (**21**) by the loss of carbon monoxide. The mono-acetate (**12**) also reveals the same fragmentation pattern as that of the compound (**4**).

Experimental

Melting points were determined on a micro hot-stage and are uncorrected. UV spectra were recorded on a Hitachi EPS-2U spectrophotometer. IR spectra were taken on a JASCO DS-701G spectrophotometer. NMR spectra were measured on a Varian T-60 and a Varian XL-100 spectrometer. MS were determined on a JEOL TMS-01SG spectrometer by use of direct sample inlet system with ionizing potential 75 eV at the lowest possible source temperature (140—180°).

The Potassium Salt (5)——To a solution of 3β,5α-diacetoxy-6-nitriminocholestane (**4**)[4] (516 mg) in methanol (125 ml) was added a solution of KOH (500 mg) in methanol (2.5 ml) and stirring was continued for 1 hr at room temperature. After removal of solvent *in vacuo*, precipitate was collected and washed with chloroform. There was obtained the potassium salt (**5**) (241 mg) as plates, mp 243—245° (from methanol). UV λ_{max}^{EtOH} mμ (ε): 244 (6200). *Anal.* Calcd. for $C_{27}H_{45}O_4N_2K \cdot H_2O$: C, 62.51; H, 9.13; N, 5.40. Found: C, 62.38; H, 9.02; N, 5.61.

3β,5α-Dihydroxy-6-nitraminocholest-6-ene (6)——To a suspension of the potassium salt (**5**) (100 mg) in chloroform (30 ml) was added 10% acetic acid (5 ml) and the reaction mixture was stirred for 1 hr at room temperature. The chloroform layer was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue (80 mg) was crystallized from methanol to give the sec-nitramine (**6**) (50 mg) as needles, mp 178—180°. UV λ_{max}^{EtOH} mμ (ε): 244 (6300). IR ν_{max}^{KBr} cm^{-1}: 3500, 3400 (OH and NH), 1580 and 1315 (NO_2). NMR (100 MHz, acetone-d_6): δ 5.88 (bs, W_H 6 Hz, 7-H), 4.00 (bs, W_H 24 Hz, 3α-H), 2.88 (bs, 2×OH and NH), 1.00 (s, 10β-Me). *Anal.* Calcd. for $C_{27}H_{46}O_4N_2$: C, 70.09; H, 10.02; N, 6.05. Found: C, 70.24; H, 10.08; N, 6.14. MS Calcd. for $C_{27}H_{46}O_4N_2$: M, 462.345. Found: M^+, 462.344.

3β,5α-Dihydroxy-6-methylnitraminocholest-6-ene (10)——To a solution of the potassium salt (**5**) (104 mg) in methanol (20 ml) was added a solution of methyl iodide (2.5 ml) in methanol (1 ml) and the reaction mixture was refluxed for 2.5 hr. After removal of solvent *in vacuo*, the residue was dissolved in chloroform. The chloroform solution was washed with aqueous $Na_2S_2O_3$ and H_2O. Removal of solvent *in vacuo* gave the residue (94 mg), whose preparative thin-layer chromatography (*Rf* 0.4) on silica gel plates (0.5 mm) using chloroform–methanol (10: 0.5 v/v) as solvent afforded the *tert*-nitramine (**10**) (42 mg) as needles, mp 187—188° (from benzene–*n*-hexane). UV λ_{max}^{EtOH} mμ (ε): 247 (7100). IR ν_{max}^{KBr} cm^{-1}: 3500, 3400 (OH), 1515 and 1290 (NO_2). NMR ($CDCl_3$): δ 5.82 (bs, W_H 5 Hz, 7-H), 4.06 (bs, W_H 24 Hz, 3α-H), 3.90 (OH), 3.47 (s, NMe), 1.57 (OH), and 0.90 (s, 10β-Me). *Anal.* Calcd. for $C_{28}H_{48}O_4N_2$: C, 70.55; H, 10.15; N, 5.88. Found: C, 70.52; H, 9.98; N, 5.97.

5α-Acetoxy-3β-hydroxy-6-nitriminocholestane (12)——A solution of the compound (**4**) (165 mg) and conc.HCl (1 ml) in tetrahydrofuran (5 ml) was allowed to stand at room temperature for 24 hr. Work-up gave the mono-acetate (**12**) (120 mg) as semi-solid whose thin-layer chromatography on silica gel using chloroform as eluent showed only one spot with *Rf* 0.2. UV λ_{max}^{EtOH} mμ (ε): 259 (3000). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1740 (OAc), 1630 (C=N), 1565 and 1310 (NO_2). NMR ($CDCl_3$): δ 3.57 (OH), 3.55 (m, 3α-H), 2.13 (s, 3β-OAc), and 0.95 (s, 10β-Me). MS Calcd. for $C_{29}H_{48}O_5N_2$–NO_2: *m/e*, 458.363. Found: $(M-NO_2)^+$, 458.362.

5α-Acetoxy-3β-*p*-bromobenzoyloxy-6-nitriminocholestane (13)——To a mixture of the mono-acetate (**12**) (114 mg) and K_2CO_3 (47 mg) in dry benzene (10 ml) was added a solution of *p*-bromobenzoyl chloride (100 mg) in dry benzene (8 ml). After reflux for 5 hr, the benzene layer was washed with H_2O. Work-up afforded a solid (189 mg) whose preparative thin-layer chromatography (*Rf* 0.7) on silica gel plates (0.5 mm)

using chloroform as solvent gave the di-ester (**13**) (69 mg) as needles, mp 173—175° (from methanol). IR ν_{max}^{KBr} cm^{-1}: 1740 (OAc), 1715 (COAr), 1630 (C=N), 1563 and 1310 (NO_2). NMR ($CDCl_3$): δ 8.07—7.47 (4× arom-H), 5.05 (bs, W_H 24 Hz, 3α-H), 2.23 (s, 3β-OAc), and 1.10 (s, 10β-Me). *Anal.* Calcd. for $C_{36}H_{51}O_6N_2Br$: C, 62.87; H, 7.48; N, 4.07. Found: C, 62.99; H, 7.47; N, 4.11.

3β-Acetoxy-5α-hydroxy-6-nitriminocholestane (**15**)——Anhydrous hydrogen chloride was introduced into a solution of 3β-acetoxy-6-hydroximinocholestan-5α-ol (**14**)[7] (204 mg) and freshly distilled *tert*-butyl nitrite (127 mg) in dry benzene (6 ml) with stirring for 1 hr at room temperature. The reaction mixture was washed with aqueous Na_2CO_3 and H_2O, and then dried over Na_2SO_4. Work-up gave the nitrimine (**15**) (197 mg) which was used for the next step without further purification. Recrystallization of the above compound from *n*-hexane gave needles of mp 190—192°. NMR ($CDCl_3$): δ 5.04 (bs, W_H 24 Hz, 3α-H), 3.50 (OH), 2.03 (s, 3β-OAc), and 0.97 (s, 10β-Me). *Anal.* Calcd. for $C_{29}H_{48}O_5N_2$: C, 69.01; H, 9.59; N, 5.55. Found: C, 69.11; H, 9.58; N, 5.53.

3β,5α-Diacetoxy-6-nitriminocholestane (**4**)——To a solution of the nitrimine (**15**) (100 mg) in acetic anhydride (0.2 ml) was added BF_3–OEt_2 (4 drops). The reaction mixture was stirred for 1.5 hr at room temperature and then extracted with benzene. The benzene layer was worked-up in the usual way to give a solid (107 mg) whose recrystallization from methanol afforded the compound (**4**) (75 mg) as needles, mp 172—174°. UV λ_{max}^{EtOH} mμ (ε): 268 (530). IR ν_{max}^{KBr} cm^{-1}: 1745, 1735 (2×OAc), 1630 (C=N), 1575 and 1310 (NO_2). NMR ($CDCl_3$): 4.85 (bs, W_H 24 Hz, 3α-H), 2.17 (s, 5α-OAc), 2.02 (s, 3β-OAc), and 1.03 (s, 10β-Me). This compound was identified with the compound obtained by the procedure described in lit. 4 on the basis of mixed mp and comparisons of spectral data.