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# Syntheses of Epimeric 2-Hydroxy-16-chloroestrone Monomethyl Ethers[1]

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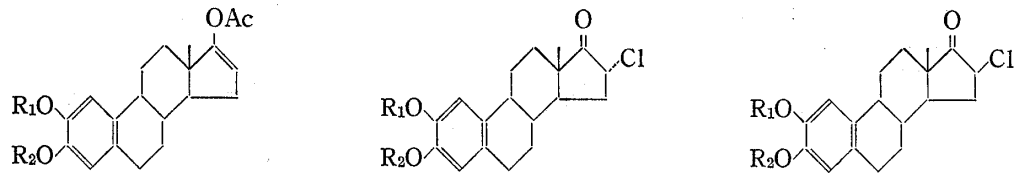
During the course of our studies on the metabolic fate of 16-chloroestrone methyl ethers in the rabbit the titled compounds have been required as authentic samples for direct comparison with the metabolites. The present paper deals with the syntheses of two pairs of C-16 epimeric 2-hydroxy-16-chloroestrone 2- and 3-monomethyl ethers.

An initial project was focused on the preparation of the 2-monomethyl ethers employing the methods worked out by Fajkoš, *et al.*[3] and Fishman, *et al.*[4] The $\Delta^{16}$-enol acetate (Ia), readily obtainable from 2-methoxyestrone benzyl ether,[5] was treated with the calculated amount of chlorine under the non-enolizing condition to yield the 16$\alpha$-chloro-17-ketone (IIa). Elimination of the benzyl group was effected by hydrogenolysis over palladium-on-charcoal resulting in formation of 2-methoxy-16$\alpha$-chloroestrone (IIb). Being adsorbed on alumina overnight, IIa was easily epimerized to afford a mixture of the thermodynamically more stable 16$\beta$-epimer (IIIa) and unchanged IIa. Separation of these two was attained by preparative thin-layer chromatography (TLC) on multiple runs to give the desired IIIa in a satisfactory yield. Removal of the protecting group at C-3 by catalytic hydrogenation provided the remaining 2-methoxy-16$\beta$-chloroestrone (IIIb).



Ia : $R_1$=$CH_3$, $R_2$=$CH_2Ph$
Ib : $R_1$=$CH_2Ph$, $R_2$=$CH_3$

IIa : $R_1$=$CH_3$, $R_2$=$CH_2Ph$
IIb : $R_1$=$CH_3$, $R_2$=H
IIc : $R_1$=$CH_2Ph$, $R_2$=$CH_3$
IId : $R_1$=H, $R_2$=$CH_3$

IIIa : $R_1$=$CH_3$, $R_2$=$CH_2Ph$
IIIb : $R_1$=$CH_3$, $R_2$=H
IIIc : $R_1$=$CH_2Ph$, $R_2$=$CH_3$
IIId : $R_1$=H, $R_2$=$CH_3$

Chart 1

Next efforts were directed to the preparation of the isomeric 3-methyl ethers. The $\Delta^{16}$-enol acetate (Ib) derived from 2-benzyloxyestrone methyl ether[5] was similarly treated with the calculated amount of chlorine in the presence of anhydrous potassium carbonate yielding the 16$\alpha$-chloro-17-ketone (IIc). Being submitted to hydrogenolysis, IIc underwent facile debenzylation to furnish 2-hydroxy-16$\alpha$-chloroestrone 3-methyl ether (IId). Epimerization of IIc by contact with alumina gave a mixture of the more stable 16$\beta$-epimer (IIIc) and unchanged IIc, which was submitted to further elaboration without purification. Subsequent

1) This paper constitutes Part LXXIII of the series entitled "Analytical Chemical Studies on Steroids"; Part LXXII: T. Nambara, Y. Matsuki, J. Igarashi, Y. Kawarada, and M. Kurata, *Chem. Pharm. Bull.* (Tokyo), **22**, 2242 (1974).
2) Location: *Aobayama, Sendai.*
3) J. Fajkoš, *Collection Czecho. Chem. Commun.*, **20**, 312, 1478 (1955); J. Fajkoš and F. Šorm, *ibid.*, **24**, 766 (1959).
4) J. Fishman and W.R. Biggerstaff, *J. Org. Chem.*, **23**, 1190 (1958).
5) T. Nambara, Y. Kawarada, M. Asama, S. Akiyama, and M. Nokubo, *Chem. Pharm. Bull.* (Tokyo), **21**, 2725 (1973).

hydrogenolysis afforded two epimeric 2-hydroxy-16-chloroestrone 3-methyl ethers, from which the desired 16$\beta$-epimer (IIId) was separated by preparative TLC on multiple runs.

It is hoped that the availability of these synthetic samples may be helpful for structural elucidation of the metabolites formed from 16-chloroestrone methyl ethers.

## Experimental[6]

**2-Methoxy-3-benzyloxy-16$\alpha$-chloroestra-1,3,5(10)-trien-17-one (IIa)**——To a solution of 2-methoxy-3-benzyloxyestra-1,3,5(10),16-tetraen-17-ol acetate (Ia)[5] (500 mg) in $CCl_4$ (25 ml) containing anhydrous $K_2CO_3$ (660 mg) the calculated amount of $Cl_2$ dissolved in $CCl_4$ was added dropwise with stirring at 0°. The reaction mixture was filtered, diluted with ether, washed with 0.1N $Na_2S_2O_3$ and $H_2O$, and dried over anhydrous $Na_2SO_4$. After usual work-up the crude product was recrystallized from MeOH to give IIa (260 mg) as colorless needles. mp 145.5—146.5°. $[\alpha]_D^{26}$ +126° ($c$=0.15). *Anal.* Calcd. for $C_{26}H_{29}O_3Cl$: C, 73.48; H, 6.88. Found: C, 73.13; H, 6.87. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1758 (C=O). NMR (4% solution in $CCl_4$) $\delta$: 0.90 (3H, s, 18-$CH_3$), 3.73 (3H, s, 2-$OCH_3$), 4.25 (1H, t, $J$=3.9 Hz, 16$\beta$-H), 4.92 (2H, s, 3-$OCH_2Ph$), 6.48 (1H, s, 4-H), 6.65 (1H, s, 1-H), 7.26 (5H, m, 3-$OCH_2C_6\underline{H}_5$).

**2-Methoxy-3-hydroxy-16$\alpha$-chloroestra-1,3,5(10)-trien-17-one (IIb)**——A solution of IIa (100 mg) in EtOH (10 ml)–AcOEt (10 ml) was shaken with 10% Pd/C (50 mg) under a stream of $H_2$ gas for 6 hr. After usual work-up the crude product obtained was submitted to preparative TLC using benzene–$CHCl_3$–AcOEt (10: 2: 1) as developing solvent. Elution of the adsorbent corresponding to the spot ($Rf$ 0.75) with AcOEt and recrystallization of the eluate from hexane gave IIb (56 mg) as colorless needles. mp 104—107°. $[\alpha]_D^{27}$ +140° ($c$=0.11). *Anal.* Calcd. for $C_{19}H_{23}O_3Cl$: C, 68.15; H, 6.92. Found: C, 68.04; H, 6.93. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1750 (C=O). NMR (4% solution in $CDCl_3$) $\delta$: 0.93 (3H, s, 18-$CH_3$), 3.81 (3H, s, 2-$OCH_3$), 4.38 (1H, t, $J$= 3.9 Hz, 16$\beta$-H), 5.31 (1H, s, 3-OH), 6.55 (1H, s, 4-H), 6.68 (1H, s, 1-H).

**2-Methoxy-3-benzyloxy-16$\beta$-chloroestra-1,3,5(10)-trien-17-one (IIIa)**——IIa (50 mg) was dissolved in benzene, adsorbed on $Al_2O_3$ (800 mg), and allowed to stand overnight. Elution with AcOEt gave an oily product, which in turn was submitted to preparative TLC by multiple runs using $CHCl_3$–hexane (1: 1) as developing solvent. Elution of the adsorbent corresponding to the spot with AcOEt and recrystallization of the eluate from MeOH gave IIIa (25 mg) as colorless needles. mp 167—169°. $[\alpha]_D^{17}$ +90° ($c$=0.15). *Anal.* Calcd. for $C_{26}H_{29}O_3Cl$: C, 73.48; H, 6.88. Found: C, 73.26; H, 6.95. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1752 (C=O). NMR (2% solution in $CCl_4$) $\delta$: 1.07 (3H, s, 18-$CH_3$), 3.79 (3H, s, 2-$OCH_3$), 4.98 (2H, s, 3-$OCH_2Ph$), 6.51 (1H, s, 4-H), 6.69 (1H, s, 1-H), 7.30 (5H, m, 3-$OCH_2C_6\underline{H}_5$).

**2-Methoxy-3-hydroxy-16$\beta$-chloroestra-1,3,5(10)-trien-17-one (IIIb)**——A solution of IIIa (30 mg) in EtOH (10 ml)–AcOEt (10 ml) was shaken with 10% Pd/C (50 mg) under a stream of $H_2$ gas for 2 hr. After usual work-up the crude product obtained was submitted to preparative TLC by multiple runs using $CHCl_3$ as developing solvent. Elution of the adsorbent corresponding to the spot with AcOEt and recrystallization of the eluate from $CCl_4$ gave IIIb (20 mg) as colorless needles. mp 162—165°. $[\alpha]_D^{20}$ +131° ($c$=0.13). *Anal.* Calcd. for $C_{19}H_{23}O_3Cl \cdot 1/2H_2O$: C, 66.37; H, 7.04. Found: C, 66.33, 66.36; H, 7.29, 7.27. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1758 (C=O). NMR (1.6% solution in $CCl_4$) $\delta$: 1.05 (3H, s, 18-$CH_3$), 3.81 (3H, s, 2-$OCH_3$), 5.13 (1H, s, 3-OH), 6.48 (1H, s, 4-H), 6.59 (1H, s, 1-H).

**2-Benzyloxy-3-methoxy-16$\alpha$-chloroestra-1,3,5(10)-trien-17-one (IIc)**——To a solution of 2-benzyloxy-3-methoxyestra-1,3,5(10),16-tetraen-17-ol acetate (Ib)[5] (110 mg) in $CCl_4$ (20 ml) containing anhydrous $K_2CO_3$ (150 mg) the calculated amount of $Cl_2$ dissolved in $CCl_4$ was added dropwise with stirring at 0°. The reaction mixture was filtered, diluted with ether, washed with 0.1N $Na_2S_2O_3$ and $H_2O$, and dried over anhydrous $Na_2SO_4$. After usual work-up the crude product was submitted to preparative TLC using benzene as developing solvent. Elution of the adsorbent corresponding to the spot ($Rf$ 0.65) with AcOEt and recrystallization of the eluate from MeOH gave IIc (80 mg) as colorless needles. mp 162—163°. $[\alpha]_D^{26}$ +115° ($c$=0.10). *Anal.* Calcd. for $C_{26}H_{29}O_3Cl$: C, 73.48; H, 6.88. Found: C, 73.49; H, 6.86. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1759 (C=O). NMR (4% solution in $CCl_4$) $\delta$: 0.90 (3H, s, 18-$CH_3$), 3.75 (3H, s, 3-$OCH_3$), 4.27 (1H, t, $J$= 3.9 Hz, 16$\beta$-H), 4.92 (2H, s, 2-$OCH_2Ph$), 6.45 (1H, s, 4-H), 6.68 (1H, s, 1-H), 7.27 (5H, m, 2-$OCH_2C_6\underline{H}_5$).

**2-Hydroxy-3-methoxy-16$\alpha$-chloroestra-1,3,5(10)-triene-17-one (IId)**——A solution of IIc (13 mg) in EtOH (5 ml)–AcOEt (5 ml) was shaken with 10% Pd/C (10 mg) under a stream of $H_2$ gas for 30 min. After usual work-up the crude product obtained was submitted to preparative TLC using benzene–$CHCl_3$–AcOEt (10: 2: 1) as developing solvent. Elution of the adsorbent corresponding to the spot ($Rf$ 0.75) with AcOEt

6) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$. Infrared (IR) spectra were run on JASCO Model IRA-1 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on Hitachi Model R-20A spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, t=triplet, and m=multiplet. For preparative TLC silica gel $HF_{254}$ (E. Merck AG, Darmstadt) was used as an adsorbent.

and recrystallization of the eluate from benzene gave IId (10 mg) as colorless needles. mp 171—173°. $[\alpha]_D^{19}$ +50° ($c$=0.11). *Anal.* Calcd. for $C_{19}H_{23}O_3Cl \cdot C_6H_6$: C, 72.71; H, 7.08. Found: C, 72.82; H, 6.93. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1758 (C=O). NMR (1.6% solution in $CDCl_3$) $\delta$: 0.95 (3H, s, 18-$CH_3$), 3.82 (3H, s, 3-$OCH_3$), 4.38 (1H, t, $J$=3.9 Hz, 16$\beta$-H), 5.31 (1H, s, 3-OH), 6.49 (1H, s, 4-H), 6.78 (1H, s, 1-H).

**2-Hydroxy-3-methoxy-16$\beta$-chloroestra-1,3,5(10)-trien-17-one (IIId)**——IIc (50 mg) was dissolved in benzene, adsorbed on $Al_2O_3$ (100 mg), and allowed to stand overnight. Elution with benzene gave a mixture of two C-16 epimers as colorless oil. A solution of this crude product was shaken with 10% Pd/C (40 mg) under a stream of $H_2$ gas for 2 hr. After usual work-up the crude product obtained was submitted to preparative TLC by multiple runs using hexane–$CHCl_3$ (1:1) as developing solvent. Elution of the adsorbent corresponding to the spot with AcOEt and recrystallization of the eluate from MeOH gave IIId (20 mg) as colorless needles. mp 175—177°. $[\alpha]_D^{16}$ +120° ($c$=0.10). *Anal.* Calcd. for $C_{19}H_{23}O_3Cl \cdot 1/2H_2O$: C, 66.37; H, 7.04. Found: C, 66.21, 66.12; H, 6.86, 6.91. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1753 (C=O). NMR (5% solution in $CCl_4$) $\delta$: 1.02 (3H, s, 18-$CH_3$), 3.80 (3H, s, 3-$OCH_3$), 5.17 (1H, s, 2-OH), 6.39 (1H, s, 4-H), 6.66 (1H, s, 1-H).

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# Studies on Berberine Derivatives and Related Alkaloids. VII.[1] On the Biosynthesis of Protopine[2]

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Contrary to our expectations, tetrahydrocoptisine methochloride (III A) was not incorporated into corynoline.[4] We further examined the fate of III A and found that this alkaloid was actually incorporated into protopine (I) on which we describe in this paper.

Incorporation of (+)-reticuline, (—)-scoulerine, stylopine and cheilanthifoline besides S-methyl group of L-methionine into protopine (I) have already been reported.[5] However, there was no evidence of the incorporation of tetrahydrocoptisine N-methyl derivative (III) into protopine (I).

(N-$^{14}CH_3$)-tetrahydrocoptisine methochloride (III A) was administered to *Corydalis incisa* in May by the cotton wick method and radioactive protopine (I) was obtained through the usual work up, 0.19% incorporation.

In order to prove the labelled position of protopine (I), the alkaloid was reduced with $LiAlH_4$ to give dihydroprotopine (II), which was heated with 20% HCl yielding tetrahydrocoptisine methochloride (III A).

1) Part VI: C. Tani, S. Takao and K. Tagahara, *Yakugaku Zasshi*, 93, 197 (1973).
2) This work was presented at the 21st Annual Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka, November, 1971, Abstracts of Papers, p. 25.
3) Location: *Motoyamakita-cho, Higashinada-ku, Kobe.*
4) This work was presented at the 91th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April, 1971, Abstracts of Papers, p. 727.
5) M. Sribney and S. Kirkwood, *Nature*, 71, 931 (1953); D.H.R. Barton, R.H. Hesse and G.W. Kirby, *J. Chem. Soc.*, 1965, 6379; A.R. Battersby, R.J. Francis, M. Hirst, R. Southgate and J. Staunton, *Chem. Commun.*, 1967(12), 602; N. Takao and K. Iwasa (This work was presented at the 91th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April, 1971, Abstracts of Papers, p. 727.