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TRANSFORMATION OF KURCHI ALKALOIDS—III*

SOME NEIGHBOURING GROUP EFFECTS IN DERIVATIVES OF HOLARRHIMINE

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Abstract—By a selective protection of the 3-amino group of holarrhimine by acetylation, derivatives of holarrhimine involving the 18,20 carbon atoms were obtained. 3β-Hydroxy-18,20β-oxido-5-pregnene and its nitrate ester obtained previously by the action of nitrous acid on holarrhimine were synthesized by an independent route. 18-Hydroxyprogesterone was obtained in yields of 45–50% in a single stage oxidation by the Ruschig procedure.

THE presence of three reactive functional groups in holarrhimine[1,2] (18-hydroxy-3β, 20α-diamino-5-pregnene; Ia) often presents difficulties in many reactions. The amino groups differ in their reactivities to most reagents and the presence of the 18-hydroxyl group vicinal to the 20α-amino group leads to intramolecular reactions, especially with reagents such as nitrous acid.[3]

A method for selective protection of the functional group of holarrhimine was necessary. In this respect, the proximity of the 20-amino group and the 18-hydroxyl also presents an advantage. Sorm *et al.*[4] ingeniously exploited this proximity relationship to protect the amino group at position 20 selectively by a base-catalyzed acyl migration from the oxygen at position 18. In the present work advantage was taken of a selective hydrolytic removal of an acyl group from the nitrogen at position 20 by an anchimeric assistance from the neighbouring 18-hydroxyl. It was thus possible to isolate the 20α-amino-18-hydroxy system for studying some neighbouring group effects.

Starting from triacetylholarrhimine (Ib) the neutral 3,20-diacetyl derivative (Ic) was obtained by selective hydrolysis under mild alkaline conditions. The free hydroxymethyl group in Ic was oxidized to the corresponding 18-aldehyde (Id) and the 18-carboxylic acid (Ie) by chromium trioxide. The 18-O-mesylate of Ic(If) was obtained by treatment with methanesulfonyl chloride in pyridine, but the yields were poor and a projected synthesis of progesterone through this route was considered unprofitable.

On mild acid hydrolysis diacetylholarrhimine was converted to the basic 3-monoacetyl derivative (Ig). In this reaction anchimeric assistance was presumably

* N.C.L. Communication No. 539.

[1] S. Siddiqui and P. P. Pillay, *J. Ind. Chem. Soc.* **9**, 553 (1932).
[2] L. Lábler, V. Cerny and F. Sorm, *Chem. Ind.* 1119 (1955).
[3] Mansa Ram, D. D. Godse and P. K. Bhattacharyya, *Tetrahedron*, in press.
[4] L. Lábler and F. Sorm, *Chem. Ind.* 598 (1959).

obtained from the 18-hydroxyl function possibly via hydrogen bonding interaction between the amide nitrogen and the 18-oxygen. The 3β-acetamido group is on the other hand relatively resistant to the action of mild acid or alkali.

Earlier experience on the action of nitrous acid on holarrhimine[3] had indicated that a majority of the isolated products had a 18-20β-ether linkage. This is in contrast with the observed behavior[5] of 18-unsubstituted 20-aminosteroids which give rise to a mixture of 20-hydroxy compound and $\Delta^{17,20}$ and Δ^{20-21} elimination products with nitrous acid. Nitrous acid reaction on compound Ig gave 3β-acetamido-18,20β-oxido-5-pregnene (IIa) as a major product. In fact, no other product could be isolated in significant amounts from the reaction mixture. It is interesting to compare in this connection the behaviours of 18-amino-20β-hydroxysteroids which have been recently shown to undergo Demjanov rearrangement to the 13,14-cyclopregnanes by Dauben and Lang and Sorm̌ *et al.*[6] Presumably in the case of 18-aminosteroids the ease of formation of the non-classical cation (Fig. 1) may be a determining factor.

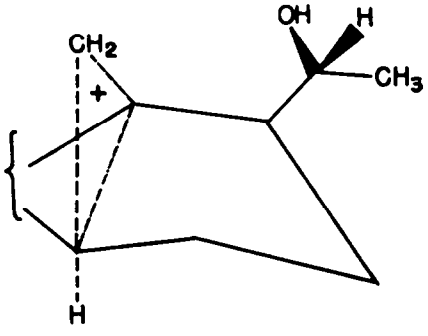


FIG. 1.

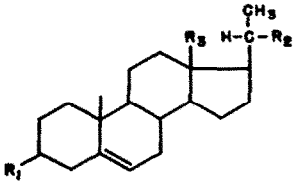
Drastic alkaline hydrolysis of compound IIa gave the free base, IIb, which was obtained previously in this Laboratory[3] by the lithium aluminium hydride reduction of IIe. On nitrous acid treatment compound IIb gave a mixture of three products (as detected by paper chromatography).[3] On chromatography over alumina two of these were isolated in pure form and identified as the 3β-hydroxy-18,20β-oxido-5-pregnene (IIc) and its nitrate ester (IId) through their physical constants and comparative IR spectra with authentic samples.[3]

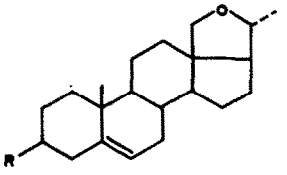
Deamination by the Ruschig procedure of compound Ig gave 18-hydroxy-3β-acetamide-20-oxo-5-pregnene (IIIa) which existed exclusively in the hemiketal form (absence of 20-carbonyl band in the I.R. spectra) and could be converted into the corresponding methyl ketal (IIIb) by treatment with *p*-toluenesulfonic acid in methanol. Holarrhimine itself was also converted into the known 18-hydroxyprogesterone (IV)[4,7,8] by Ruschig procedure. Sorm̌ *et al.*[4] had also reported the conversion of holarrhimine to 18-hydroxyprogesterone in five stages by a different route. Simultaneous deamination of both the amino groups presented some difficulties in preliminary

[5] L. H. Sarett, *J. Biol. Chem.* **68**, 2478 (1946).
[6] W. G. Dauben and P. Lang, *Tetrahedron Letters* **11**, 453 (1962). J. Horce, V. Cerny and F. Sorm̌, Tetrahedron Letters **12**, 501 (1962).
[7] F. Buzzetti, W. Wicki, J. Kalvoda and O. Jeger, *Helv. Chim. Acta*, **42**, 388 (1959).
[8] R. Pappo, *J. Amer. Chem. Soc.* **81**, 1010 (1959).

experiments due to the formation of N-dichloro derivatives along with the expected bismonochloroderivative (Ii) during the N-chlorination reaction. By careful uniform addition of N-chlorosuccinimide to holarrhimine under vigorous agitation it was possible to increase the ultimate yield of 18-hydroxyprogesterone (IV) to 50%. Compound IV was obtained in two distinct forms one melting at 162° and the other at 188°. Both forms had identical rotation and identical I.R. spectra which indicated that they exclusively existed in the hemiketal form. But a polarity difference enabled their separation by column chromatography. However, the higher melting form could be converted to the lower melting form by repeated recrystallization from aqueous acetone or methyl ethyl ketone. The identity of compound IV was established by a comparison of its physicochemical behaviour, mixed m.p. and IR spectra with 18-hydroxyprogesterone synthesized from conessine according to the procedure of Jeger *et al.*[7]

The synthesis of 20-N-methylholarrhimine (Ii) first isolated by Tschesche and Wiensz[9] from kurchi was attempted through the 20-N-dimethyl intermediate (Ij) obtained by methylation of compound Ig by formaldehyde and formic acid. Compound Ij was also obtained in quantitative yields from Ic indicating that even under a mild acid condition an intramolecular deacylation occurs in the 18-hydroxy-20-acetamido system. On treatment with cyanogen bromide on the 18-O-acetyl derivative of Ij (Ik) the expected 20-N-cyano-N-methyl-3β-acetamido-5-pregnene was obtained (ν_{max} 2230 cm^{-1}). However all attempts at decarboxylation





Ia: $R_1 = R_2 = NH_2$; $R_3 = CH_2OH$

Ib: $R_1 = R_2 = NHAc$; $R_3 = CH_2OAc$

Ic: $R_1 = R_2 = NHAc$; $R_3 = CH_2OH$

Id: $R_1 = R_2 = NHAc$; $R_3 = CHO$

Ie: $R_1 = R_2 = NHAc$; $R_3 = COOH$

If: $R_1 = R_2 = NHAc$; $R_3 = CH_2OSO_2CH_3$

Ig: $R_1 = NHAc$; $R_2 = NH_2$; $R_3 = CH_2OH$

Ih: $R_1 = NHAc$; $R_2 = NHCl$; $R_3 = CH_2OH$

Ii: $R_1 = R_2 = NHCl$; $R_3 = CH_2OH$

Ij: $R_1 = NHAc$; $R_2 = N(CH_3)_2$; $R_3 = CH_2OH$

Ik: $R_1 = NHAc$; $R_3 = N(CH_3)_2$; $R_3 = CH_2OAc$

Il: $R_1 = NHAc$; $R_2 = N(CN)CH_3$; $R_3 = CH_2OAc$

Im: $R_1 = NH_2$; $R_2 = NHCH_3$; $R_3 = CH_2OH$

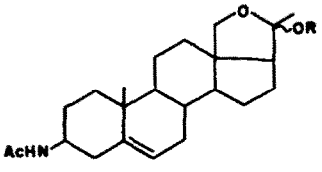
IIa: R = NHAc

IIb: $R = NH_2$

IIc: R = OH

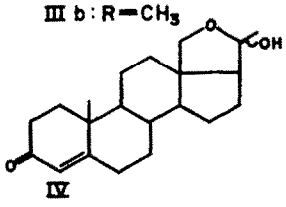
IId: $R = ONO_2$

IIe: $R = NO_2$



IIIa: R = H

IIIb: $R = CH_3$



IV

FIG. 2. Formula chart

[9] R. Tschesche and K. Wiensz, *Ber. Dtsch. Chem. Ges.* **91**, 1504 (1958).

of the N-carboxy derivative obtained after saponification failed either in acidic or alkaline conditions. Presumably the neighbouring 18-hydroxyl group interfered in some way with the decarboxylation reaction.

EXPERIMENTAL

All m.p. are uncorrected. The U.V. spectrum of 18-hydroxyprogesterone was taken in ethanol and the rotations in chloroform (unless otherwise specified) using a 1 dm tube. The I.R. spectra were obtained in Nujol on a Grubb–Parson double beam spectrophotometer or on a Perkin-Elmer Infracord.

Holarrhimine was prepared by the method described before.[3] Conessine was isolated by a modification of the method of Siddiqui and Pillay,[1] where the petroleum ether mother liquors from the precipitated carbonates were subjected to chromatography on alumina.

Holarrhimine triacetate (Ib). Holarrhimine (1·2 g) in pyridine (5·0 ml) was treated with freshly distilled acetic anhydride (3·0 ml) in the cold. The mixture was allowed to warm up to room temp and kept for 24 hr. Ice cold water (100 ml) was added to the reaction mixture with vigorous shaking and the separated solid was filtered, washed with water and crystallized from a mixture of methanol–acetone, m.p. 249°, lit.[8] 249°. (Found: C, 68·1; H, 9·2; Acetyl, 27·4. Calc. for $C_{37}H_{62}O_4N_2,H_2O$: C, 68·1; H, 9·2, Acetyl 27·3%) I.R. ν_{max} 1740, 1238 cm^{-1} (O–Ac), 3220, 1635, 1558 cm^{-1} (NHAc).

Diacetyl holarrhimine (18-*hydroxy*-3β,20α-*diacetamido*-5-*pregnene;* Ic). Triacetyl holarrhimine (1·5 g) in ethanol (80 ml) was refluxed after the addition of 60 ml of ethanolic potassium hydroxide solution (0·1 N) for 4 hr. The reaction mixture after the addition of 200 ml water was repeatedly extracted in chloroform (3 × 100 ml). The chloroform extract was washed with 5% aqueous sodium chloride (3 × 50 ml) to prevent solubility losses, dried (Na_2SO_4) and evaporated to dryness. The crystalline residue (1·35 g) was recrystallized from methanol–acetone, m.p. 285–286°; $[\alpha]_D$ −37·0 (ethanol, *c* 1·63); (Found: C, 72·6; H, 9·6; Acetyl, 22·0. $C_{25}H_{40}O_3N$: requires C, 72·2; H, 9·64; Acetyl 21·9%) I.R. ν_{max} 3380 cm^{-1} (OH), 1640, 1552 cm^{-1} (CO—NH).

Oxidation of diacetyl holarrhimine with chromium trioxide. Diacetyl holarrhimine (0·11 g) in acetic acid (7 ml) was treated with solution of chromium trioxide (70 mg) in acetic acid (2 ml) at room temp overnight (stirring). The reaction mixture was diluted with water (25 ml) and extracted with chloroform (3 × 10 ml). The chloroform layer was washed with water, aqueous sodium bicarbonate (5%; 3 × 10 ml) and again with water, dried and evaporated to get the crude aldehyde (Id; 92 mg) which was crystallized from hexane–methylene chloride. m.p. 189–190°; $[\alpha]_D$ −16·5 (*c*, 4·60). (Found: C, 72·2; H, 9·6. $C_{25}H_{38}O_5N_2$ requires: C, 72·4; H, 9·2%) I.R. ν_{max} 1700, 2680 cm^{-1} (CHO), 1639, 1555 cm^{-1} (—NH—CO).

The pooled bicarbonate extract was acidified and extracted with chloroform (3 × 10 ml). The chloroform extracts were washed with water, dried (Na_2SO_4) and evaporated to yield the crystalline 3β-20α-diacetamido-5-pregnene-18-oic acid (Ie; 10 mg), m.p. 220°. I.R. ν_{max} 2700, 1710 cm^{-1} (COOH), 3215, 1638, 1559 cm^{-1} (CO—NH).

Mesylation of compound Ic. Diacetyl holarrhimine (250 mg) was suspended in anhydrous pyridine (5 ml) at 0° and freshly distilled methanesulfonyl chloride (0·3 ml) was added with vigorous agitation. After keeping in the cold for 5 min the mixture was agitated on a shaker for 1 hr at room temp. The reddish brown product was poured into ice-cold aqueous sodium carbonate (5%, 20 ml) and extracted with chloroform containing 5% methanol (3 × 15 ml). The chloroform extract was washed with dil. hydrochloric acid, aqueous sodium chloride (5%), then with a little water, dried and evaporated. The residue was chromatographed over neutral grade II alumina. The mesylate (If, 15 mg) was eluted with chloroform. Recrystallized from methanol–acetone, m.p. 198–199°. (Found: S, 6·46. $C_{26}H_{42}O_5N_2S$ requires: S, 6·47%). I.R. ν_{max} 1333, 1170 cm^{-1} (S═O), 3180, 1632, 1548 cm^{-1} (NH—CO).

The yield of compound If could not be improved further by prolonged treatment of Ic with methanesulfonyl chloride.

3-*Monoacetylholarrhimine* (18-*hydroxy*-20α-*amino*-3β-*acetamido*-5-*pregnene;* Ig). Diacetyl holarrhimine (Ic, 1·1 g) in methanol (100 ml) was refluxed with 2 N sulfuric acid (80 ml) for 4 hr and the methanol was evaporated. The residual solution was cooled, treated with excess liquor ammonia and extracted with chloroform (3 × 50 ml). The chloroform extract was washed with water, dried and

evaporated to yield the crystalline monoacetyl holarrhimine (0·98 g). Recrystallized from tetrahydrofuran, m.p. 255–256°; $[\alpha]_D$ −20·7 (*c*, 1·75). (Found: C, 74·1; H, 10·2. $C_{32}H_{48}O_3N_2$ requires: C, 73·4; H, 10·2%) I.R. ν_{max} 3300 cm^{-1} (OH) 1638, 1548 cm^{-1} (CO—NH).

Compound Ig was also obtained directly from compound Ib, holarrhimine triacetate, by acid hydrolysis in near-quantitative yields.

3β-Acetamido-18,20β-oxido-5-pregnene (IIa). Compound Ig (175 mg) was dissolved in 20% aqueous acetic acid (80 ml) and treated with a saturated aqueous solution of sodium nitrite (20 ml). The precipitate obtained after 30 min was extracted in chloroform (3 × 40 ml). The chloroform extract was washed with aqueous sodium bicarbonate, water, 2% sulfuric acid, water, dried and evaporated to yield a crystalline product (147 mg). Recrystallized from benzene or methanol, m.p. 230–231°; $[\alpha]_D$ −48·7 (*c*, 1·54). (Found: C, 76·6; H, 9·8; N, 4·0. $C_{32}H_{48}O_3N$ requires: C, 77·3; H, 9·6; N, 3·9%). I.R. ν_{max} 3210 cm^{-1}, 1635, 1549 cm^{-1} (CO—NH) 1075 cm^{-1} (ether).

3β-Amino-18,20β-oxido-5-pregnene (IIb). Compound IIa (500 mg) was saponified with 30 ml of 5 N KOH in 85% ethanol in a sealed tube at 110° overnight. The mixture was diluted with water, freed from alcohol by evaporation and extracted with chloroform. The chloroform extract was washed dried and evaporated. The dry residue was dissolved in ether and the base precipitated as hydrochloride with hydrogen chloride gas. The base was liberated by alkali from an aqueous suspension of the hydrochloride and extracted in chloroform. The chloroform extract after processing in the usual manner yielded a crystalline solid (300 mg). Recrystallized from acetone, m.p. 143–144°; $[\alpha]_D$ −51° (*c*, 2·1). (Found: C, 80·61; H, 10·3. $C_{21}H_{33}NO$ requires C, 79·9; H, 10·5%). I.R. ν_{max} 3300, 1640 cm^{-1} (NH), 1080 cm^{-1} (ether).

Action of nitrous acid on compound IIb. Compound IIb (300 mg) was treated with a saturated solution of sodium nitrite (10 ml) in 20% aqueous acetic acid (30 ml) in the manner described for compound Ig. The neutral insoluble precipitate was pooled in chloroform. Paper chromatography of the crude extract[8] revealed the existence of three spots (R_f = 0·95; 0·8; 0·58). It was then chromatographed over alumina. Benzene and pet ether eluted compound IIc (46 mg). Recrystallized from methanol, m.p. 133°; mixed m.p. with nitrate ester of 3β-hydroxy-18,20β-oxido-5-pregnene[8] m.p. 133°. The I.R. spectra were identical. Compound IId (106 mg) was eluted with benzene and 5% chloroform. Recrystallized from acetone m.p. 167°, mixed with 3β-hydroxy-18,20β-oxido-5-pregnene[8] m.p. -166 167°. The I.R. spectra of the two compounds were identical.

18-Hydroxy-3β-acetamido-20-oxo-5-pregnene (18 → 20 *hemiketal;* IIIa). A 1% solution of N-chlorosuccinimide was added dropwise to a solution of 3-monoacetylholarrhimine (Ig; 288 mg) in 25 ml methylene chloride with vigorous stirring. The end point (11·1 ml of the N-chlorosuccinimide solution) was determined with bromothymol blue indicator paper. The reaction mixture was washed thoroughly free from succinimide with water, dried and concentrated *in vacuo* (bath temp 35°). The solid residue (3·0 mg) was refluxed without further purification with sodium methoxide in methanol (250 mg sodium in 15 ml absolute methanol) for 1 hr. The progress of the reaction was followed with acidified starch-iodide indicator paper. The reaction mixture was then treated with 2N sulfuric acid (25 ml) and refluxed for 1 hr. It was diluted with water (100 ml) and extracted with chloroform (3 × 50 ml). The chloroform extract was washed with water, aqueous sodium bicarbonate, then with water, dried and evaporated to yield crude compound IIIa (260 mg). Recrystallized from methanol, m.p. 174°; $[\alpha]_D$ +18·4° (*c*, 0·707). (Found: C, 73·1; H, 9·9; N, 3·7; $C_{23}H_{36}O_3N$ requires: C, 74·0; H, 9·4; N, 3·8%). Methyl ketal (IIIb) prepared from IIIa with p-toluene sulfonic acid in methanol. m.p. 288°, I.R. ν_{max} 1093, 1072 cm^{-1} (ether), 1624, 1583 cm^{-1} (—CO—NH). (Found: O—CH_3, 7·0. $C_{24}H_{38}O_3N$ requires: O—CH_3, 8·0%). On mild acid hydrolysis IIIa was regenerated from IIIb.

18-Hydroxyprogesterone (IV) *from holarrhimine.* A 8% solution of N-chlorosuccinimide in methylene chloride was added dropwise into a solution of holarrhimine (1·1 g) in methylene chloride (16 ml) with vigorous stirring. It was necessary to add the N-chlorosuccinimide at a uniform rate avoiding local excess. The end point (neutrality as indicated with a bromothymol blue indicator paper) was reached when 10·4 ml of the chlorinating solution was added. The reaction mixture was washed repeatedly with water, dried and evaporated *in vacuo* (bath temp 35°). The white crystalline product, Ii, (1·15 g) decomposed at 130°. (Found: Cl, 17·2. $C_{21}H_{34}ON_2Cl_2$ requires: Cl, 17·7%). NN'-dichloroholarrhimine (Ii, 1·09 g) was refluxed for 1 hr with methanolic sodium methoxide until the solution did not liberate iodine in acidified starch-iodide indicator paper. It was

diluted with water and extracted with chloroform. The chloroform extract was worked up in the usual manner to yield a gummy yellow residue (0·89 g) which failed to crystallize. The residue was hydrolyzed in 1N sulfuric acid in 50% aqueous ethanol (160 ml) for 1 hr. After removal of alcohol the steroid was extracted in chloroform (3 × 40 ml). The chloroform extract was washed with water, dried and evaporated to a yellowish brown gum (0·75 g) which was chromatographed over neutral alumina (Gr. II; 22 g). Benzene and benzene–methylene chloride mixtures eluted a crystalline compound (125 mg). Recrystallized from aqueous acetone, m.p. 162°, lit. 159°[7], 172–182°[8]; $[\alpha]_D$ +159 (*c*, 2·0); λ_{max} 241 mμ, log λ_{max} 4·21. (Found: C, 76·5; H, 9·1. Calc. for $C_{21}H_{30}O_3$: C, 76·3; H, 9·1%). I.R. ν_{max} 3420 cm^{-1} (OH), 1663, 1615 cm^{-1} (α,β-unsaturated C=O).

A second form of the compound was obtained from the methylene chloride eluates from the column m.p. 182° (200 mg). On crystallization from aqueous acetone this higher melting form was converted to the lower melting form (m.p. 162°).

In subsequent runs it was not necessary to chromatograph the product. The gummy residue after hydrolysis was dissolved in methyl ethyl ketone and kept for 10 days, when crystals (m.p. 159–160°) were obtained (yield from holarrhimine 45–50%).

For comparison, 18-hydroxyprogesterone was also prepared from conessine following the procedure of Buzzetti *et al.*[7] The mixed m.p. of the two products showed no depression. The I.R. spectra were identical.

18-*Hydroxy*-3β-*acetamido*-20α-N-*dimethyl*-5-*pregnene* (Ij). 3-Monoacetylholarrhimine (Ig, 300 mg) was heated on a water bath with a mixture of formic acid (98%, 1·5 ml) and formaldehyde (40%, 1·5 ml) for 4 hr. The reaction mixture was poured in water and the base liberated after basification with ammonia was extracted in chloroform (13 × 20 ml). The chloroform extract was washed with water, dried and evaporated. The crystalline residue (285 mg) was recrystallized from methanol–acetone, m.p. 246–248°; $[\alpha]_D$ −36·7 (*c*, 1·9). (Found: C, 74·6; H, 10·8; $C_{25}H_{43}O_2N_3$ requires: C, 74·6; H, 10·4%). I.R. ν_{max} 3390 cm^{-1} (OH), 1642, 1568 cm^{-1} (CO—NH). 18-O-Acetyl derivative prepared in the usual manner with pyridine–acetic anhydride. Recrystallized from acetone or benzene, m.p. 180–181° $[\alpha]_D$ −17·4° (*c*, 1·84). (Found: Acetyl, 15·4; $C_{27}H_{44}O_3N_3$ requires: Acetyl, 19·4%). I.R. ν_{max} 1735, 1265 cm^{-1} (O-Acetyl).