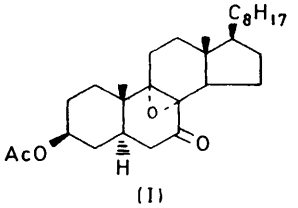
Reactions of Steroidal 8α,9α- and 8α,14α-Epoxy-7-ketones with Acetic Acid

By **Mario Anastasia,* Giuliana Cighetti, and Ada Manzocchi Soave,** Institute of Chemistry, Faculty of Medicine, University of Milan, via Saldini, 50, I-20133 Milan, Italy

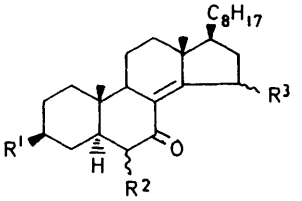
3β-Acetoxy-8α,9α-epoxy-5α-cholestan-7-one in acetic acid under reflux gave 3β-acetoxy-5α-cholesta-8,14-dien-7-one. Under the same conditions, 3β-acetoxy-8α,14α-epoxy-5α-cholestan-7-one yieded 3β,6α-, 3β,15β-, and 3β,-15α-diacetoxy-5α-cholest-8(14)-en-7-one. The mechanism of formation of these products is discussed.

PROTIC acids are known to cleave steroidal ' αβ '-epoxy-ketones (*i.e.* 1,2- and 4,5-epoxy-3-ketones [1,2] and 4,5-epoxy-6-ketones [3]), affording compounds in which the nucleophilic moiety of the acid is found at positions ' α ', ' α' ', or ' β ' [1] to the carbonyl group. The reaction sites depend on the natures of both the steroid molecule and the acid. On the other hand, steroidal 8α,9α- and 8α,14α-epoxy-7-ketones are transformed into 8,14-dienones by hydrochloric acid in boiling ethanol.[4] In this case, however, the formation of the diene system conjugated to the carbonyl group may be favoured by the presence of a fully substituted oxiran ring and the forcing conditions used.

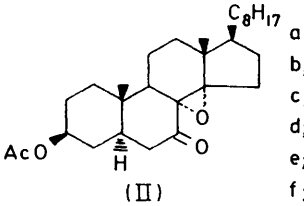
In the absence of reports of the reactions of weak acids with 8α,9α- and 8α,14α-epoxy-7-ketones, we investigated



(I)



(III)



(II)

	R^1	R^2	R^3
a;	OAc	α-OAc	H
b;	OAc	H	β-OAc
c;	OAc	H	α-OAc
d;	H	H	H
e;	OAc	H	H
f;	OAc	β-OAc	H

the reactions of the 3β-acetoxy-5α-cholestane derivatives (I) [5] and (II) [5] with boiling acetic acid. The epoxide (I) afforded the 8,14-dien-7-one,[6] as in the reaction with hydrochloric acid. However, the epoxide (II) gave products which contained an acetoxy-group not only ' α ' to the carbonyl group but also ' α ' to the double bond end of the conjugated system formed concomitantly.

As soon as the epoxide (II) had disappeared the reaction was stopped, and three isomeric products (IIIa—c) ($C_{31}H_{48}O_5$) were isolated by column chromatography. Their u.v. spectra indicated the presence in each compound of an ' α,β '-unsaturated ketone system. In addition each afforded 3β-acetoxy-5α-cholest-8(14)-en-7-one (IIIe) with zinc in boiling acetic acid. These data strongly suggested that compounds (IIIa—c) were isomeric diacetoxy-' αβ '-unsaturated ketones.

The u.v. absorption maximum of 3β,6α-diacetoxy-5α-cholest-8(14)-en-7-one (IIIa) (265 nm) was in good agreement with the value reported for $\Delta^{8(14)}$-7-ketones (262 nm), but the i.r. spectrum showed a C=O stretching band at 1 695 cm^{-1}, a higher value than that reported (1 665 cm^{-1}) for an 8(14)-en-7-one.[7] This could be accounted for by an acetoxy-group ' α ' to the carbonyl group.[8] The position and configuration of the acetoxy-group could be established from the ^1H n.m.r. spectrum. The C*H*·OAc signal was a doublet centred at δ 4.92 (*J* 12 Hz), the coupling constant being in good agreement with that for the 6β-proton of a 6α-acetoxy-7-ketone obtained from viridomicinic acid A ($J_{5\alpha,6\beta}$ 12.5 Hz).[9] The position of the C-10 methyl signal (δ 1.01) was in agreement with the value (δ 0.982) obtained by adding to the C-10 methyl value of 5α-cholest-8(14)-en-7-one (IIId) [7] the shifts due to 3β- and 6α-acetoxy-groups.[10] The structure (IIIa) was also supported by the mass spectrum; the most abundant peak (*m/e* 300) is considered to be due to the loss of the fragment C_2H_3–C_8H_{17} from the ion at *m/e* 440 (M^+ — AcOH). The ion at *m/e* 300 probably originates from the fission of the 13,17- and 15,16-bonds, both allylic to the 8,14-double bond. The removal of the 6α-acetoxy-group by zinc in acetic acid to give the monoacetate (IIIe) [11] is analogous to the reactions of other ' α '-acetoxy-ketones.[12]

3β,15β-Diacetoxy-5α-cholest-8(14)-en-7-one (IIIb) showed a u.v. absorption maximum (256 nm), in agreement with that (257 nm) reported for 3β,15ξ-diacetoxy-5α-ergosta-8(14),22-dien-7-one.[13] The high frequency

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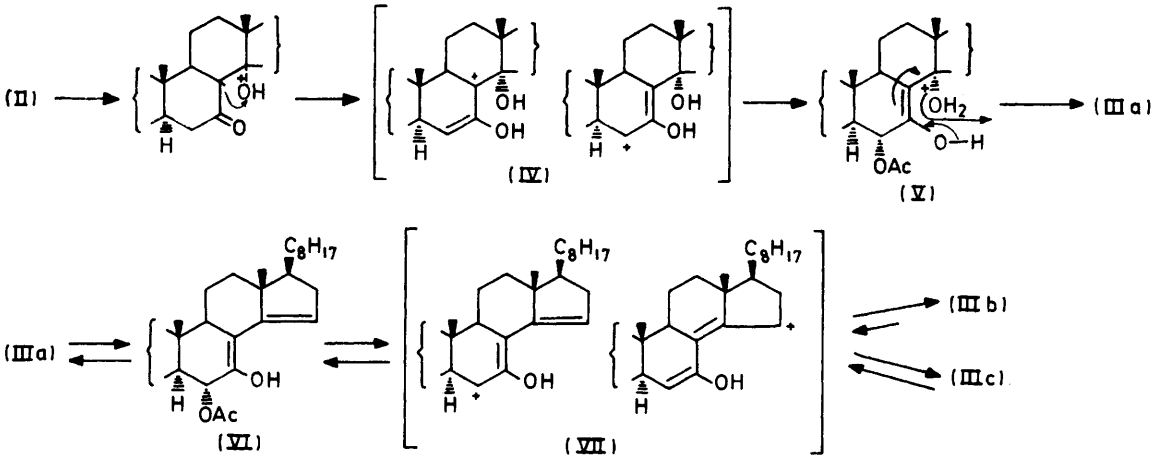
shift of the C=O stretching band (1 688 cm^{-1}) would be expected to result from an adjacent acetoxy-group. The ^1H n.m.r. spectrum showed a multiplet at δ 6.06 ($W_{1/2}$ *ca.* 15 Hz), attributable to C:C·C*H*·OAc. The low field position of this signal showed that the proton was in the same plane as the carbonyl group. The high field position of the acetoxy-signal (δ 1.91) indicated that the methyl group was shielded by the ketone.[14] Models show that such a steric arrangement exists in 3β,15β-diacetoxy-5α-cholest-8(14)-en-7-one (IIIb). The alternative structure (IIIf) is excluded because in this case the 6α-proton would exhibit only a small vicinal coupling: in fact the 6α-H in the 6β-acetoxy-7-ketone system in good agreement with the observed value (δ 0.86). The mass spectra of (IIIb) and (IIIc) differed only in the relative intensity of the most abundant ion, *m/e* 458 ($M^+ - 42$), indicating that the compounds are diastereoisomers. Treatment of (IIIc) or (IIIb) with boiling ethanol–35% hydrochloric acid yielded, after acetylation 3β-acetoxy-5α-cholest-8,14-dien-7-one.[18] Moreover alkaline hydrolysis of (IIIc) yielded a 7,15-dione identical with that obtained from (IIIb) under the same conditions.

The formation of the diacetate (IIIa) from the epoxide (II) may be rationalized[2,3] on the basis of a polar intermediate (IV), formed by initial protonation of the



SCHEME

of helvolic acid gives rise to a sharp singlet.[15] From the chemical shift of the C-13 methyl signal of (IIId)[7] (δ 0.79) it is possible to calculate by additivity rules[10] a chemical shift of δ 1.023 for the C-13 methyl of (IIIb), in good agreement with the found value (δ 1.03). The formation of the monoacetate (IIIe)[16] with zinc in boiling acetic acid confirmed structure (IIIb). Moreover, alkaline hydrolysis of (IIIb) yielded a 7,15-dione * ($\nu_{max.}$ 1 735 and 1 708 cm^{-1}); this reaction is probably similar to that of an 11-hydroxy-8-en-7-one with Triton B, which leads to a 7,11-dione.[17]

Compound (IIIc) showed a u.v. absorption maximum (257 nm) characteristic of a 15-acetoxy-8(14)-en-7-one[13] and an i.r. band at 1 680 cm^{-1}, possibly owing to the influence of an acetoxy-group near the carbonyl group. The ^1H n.m.r. spectrum exhibited a multiplet (δ 5.87; $W_{1/2}$ *ca.* 9 Hz), attributed to C*H*·OAc. On this basis the compound was identified as 3β,15α-diacetoxy-5α-cholest-8(14)-en-7-one. The calculated chemical shift of the C-13 methyl group (δ 0.865)[7,10] was

epoxide. The subsequent cleavage, depicted in the Scheme,[19] is facilitated by relief of the strain due to the 8α-substitution. Subsequent attack of acetic acid or its nucleophilic counterpart, acetate ion, at the electrophilic C-6 yields the intermediate (V), from which compound (IIIa) originates by loss of water from C-14. Most likely (IIIb) and (IIIc) originate from the enol (VI). A direct, concerted attack of acetate on (VI) cannot be excluded, although intermediate formation of the ion (VII) seems more probable. This reaction formally resembles the reverse of the transformation of 6β-bromocholest-4-en-3-one into 2α-acetoxycholest-4-en-3-one.[20] To confirm the proposed mechanism compounds (IIIa—c) were refluxed in acetic acid for 1 h. Compound (IIIb) was unchanged, whereas (IIIa) was transformed partially into (IIIb) and (IIIc) and compound (IIIc) afforded in part (IIIa) and (IIIb); this behaviour indicates that (IIIb) is more stable than (IIIa) or (IIIc). In addition models show that in the

* The small amount of this compound has not allowed us to establish the stereochemistry at C-8 and C-14.

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cases of (IIIa) and (IIIc) but not (IIIb) the new acetoxy-group interacts strongly with the 7-carbonyl group.

EXPERIMENTAL

I.r. spectra were obtained for solutions in chloroform with a Perkin-Elmer 257 spectrometer. ^{1}H N.m.r. spectra were determined with deuteriochloroform as solvent and tetramethylsilane as internal reference with a Varian HA-100 spectrometer. Routine optical rotations were recorded with a Perkin-Elmer 141 spectropolarimeter for solutions in chloroform. Mass spectra were determined with an LKB 9000 gas chromatograph–mass spectrometer operating at 20 eV by use of the direct inlet system. The progress of all reactions and column chromatography was monitored by t.l.c. on silica gel (HF_{254}) microplates and g.l.c. (2 m silanized glass column of 3% SE 30 on GasChrom Q, operating at 240—260 °C). Column chromatography was carried out on silica gel G–Celite (50 : 50 v/v) with benzene as eluant.

Treatment of 3β-*Acetoxy*-8α,9α-*epoxy*-5α-*cholestan*-7-*one* (I) *with Acetic Acid.*—A solution of the epoxide (I) (1.0 g) in glacial acetic acid (100 ml) was refluxed for 12 h under nitrogen, cooled, diluted with water, and extracted with diethyl ether. The extract was evaporated and the residue (980 mg) was crystallized twice from methanol to give 3β-acetoxy-5α-cholesta-8,14-dien-7-one (590 mg), m.p. 173—174°, $[\alpha]_D^{20}$ −16°, $\lambda_{max.}$ 224 (ε 15 200) and 298 nm (4 900) {lit.,[6] m.p. 172—174.5°, $[\alpha]_D$ −14.5°, $\lambda_{max.}$ 224 (ε 15 600) and 298 nm (5 060)} (Found: C, 79.1; H, 10.35. Calc. for $C_{29}H_{44}O_3$: C, 79.05; H, 10.05%).

Treatment of 3β-*Acetoxy*-8α,14α-*epoxy*-5α-*cholestan*-7-*one* (II) *with Acetic Acid.*—A solution of the epoxide (II) (1.0 g) in glacial acetic acid (100 ml) was refluxed for 2 h under nitrogen, cooled, diluted with water, and extracted with diethyl ether. Work-up yielded a pale yellow oil (1.0 g) which was chromatographed. The combined fractions containing the least polar product was concentrated and the residue (290 mg) was crystallized from methanol to give 3β,6α-*diacetoxy*-5α-*cholest*-8(14)-*en*-7-*one* (IIIa) (208 mg), m.p. 132—133°, $[\alpha]_D^{21}$ +5.64°, $\nu_{max.}$ 1 608, 1 695, 1 732, and 1 742 cm^{-1}, $\lambda_{max.}$ 265 nm (ε 11 700); δ 0.91 (3 H, s, 13β-Me), 1.01 (3 H, s, 10β-Me; calc.[7,10] 0.982), 2.02 (3 H, s, 3β-OAc), 2.15 (3 H, s, 6α-OAc), and 4.92 (1 H, d, *J* 12 Hz, 6β-H); *m/e* 440 (*M* − AcOH) and 300 (Found: C, 74.0; H, 9.5. $C_{31}H_{48}O_5$ requires C, 74.35; H, 9.65%).

The combined fractions containing the more polar product was concentrated and the residue (490 mg) was twice crystallized from methanol–water to give the 3β,15β-*diacetate* (IIIb) (310 mg), m.p. 112—114°, $[\alpha]_D^{21}$ −56.8°, $\nu_{max.}$ 1 605, 1 688, 1 730, and 1 740 cm^{-1}, $\lambda_{max.}$ 256 nm (ε 10 800), δ 0.91 (3 H, s, 10β-Me), 1.03 (3 H, s, 13β-Me; calc.[7,10] 1.023), 1.91 (3 H, s, 15β-OAc), 2.00 (3 H, s, 3β-OAc), and 6.06 (1 H, m, $W_{1/2}$ *ca.* 15 Hz, 15α-H); *m/e* 500 (*M*) and 458 (*M* − CH_2CO) (Found: C, 74.1; H, 9.4%).

The most polar fractions from the column were combined and evaporated and the residue (190 mg) was crystallized from diethyl ether to yield the 3β,15α-*diacetate* (IIIc) (120 mg), m.p. 170—171°, $[\alpha]_D^{21}$ −65.6°, $\nu_{max.}$ 1 605, 1 680, 1 730, and 1 740 cm^{-1}, $\lambda_{max.}$ 257 nm (ε 9 890), δ 0.86 (3 H, s, 13β-H; calc.,[7,10] 0.865), 0.89 (3 H, s, 10β-H), 1.98 (3 H, s, 15α-OAc), 2.02 (3 H, s, 3β-OAc), and 5.87 (1 H, m, $W_{1/2}$ *ca.* 9 Hz, 15β-H); *m/e* 500 (*M*) and 458 (*M* − CH_2CO) (Found: C, 74.6; H, 9.7%).

Reduction of the Diacetates (IIIa—c) *with Zinc in Acetic Acid.*—In each case the steroid (500 mg) in glacial acetic acid (50 ml) was refluxed with zinc dust (1 g) for 1 h. The crude product was crystallized from methanol to give 3β-acetoxy-5α-cholest-8(14)-en-7-one (IIIe), m.p. 141—142°, identical with an authentic sample [11] (mixed m.p. and n.m.r. and mass spectra).

Treatment of the Diacetates (IIIa—c) *with Boiling Acetic Acid.*—The diacetate (IIIa) (500 mg) was boiled in acetic acid (50 ml) for 2 h. After the usual work-up, column chromatography, and crystallization, (IIIa) (115 mg), (IIIb) (150 mg), and (IIIc) (50 mg) were obtained. Under the same conditions (IIIb) was unchanged, and (IIIc) yielded (IIIc) (60 mg), (IIIb) (140 mg), and (IIIa) (110 mg).

Treatment of the Diacetates (IIIb *and* c) *with Alkali.*—The diacetate (IIIb or c) (50 mg) was dissolved in methanolic 2N-potassium hydroxide (5 ml). After 4 h the methanol was evaporated off, and the residue was dissolved in diethyl ether. The ethereal solution was washed with water, dried, and evaporated. An oily product was obtained by preparative t.l.c. which exhibited i.r. bands at 1 708 (six-membered cyclic ketone) and 1 735 cm^{-1} (five-membered cyclic ketone). After acetylation the compound showed a molecular weight (equivalent to $C_{29}H_{46}O_4$; mass spectrometry) in accord with that of a 3β-acetoxy-5α,8ξ,14ξ-cholestane-7,15-dione.

Treatment of the Diacetates (IIIb *and* c) *with Hydrochloric Acid.*—A solution of (IIIb or c) (200 mg) in 95% ethanol (20 ml) containing 36% hydrochloric acid (1 ml) was refluxed for 3 h. Work-up in the usual way afforded a product which was acetylated to yield 3β-acetoxy-5α-cholesta-8,14-dien-7-one (identified by m.p., mixed m.p., and rotation).

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