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Studies on Syntheses of Epoxycardenolides and on Their Cleavage. IV.[1] Cleavage of 3β-Hydroxy-14β,15β-epoxy-5β,17α-card-20(22)-enolide with Hydrochloric Acid or Hydrogen Chloride[2]

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Cleavage of 3β-hydroxy-14β, 15β-epoxy-5β, 17α-card-20(22)-enolide (Ia) or its acetate (Ib) with hydrochloric acid in methanol or hydrogen chloride in chloroform resulted in the formation of 14β, 15β-dihydroxy-17α-cardenolide (III) and 14β-chloro-15β-hydroxy-17α-cardenolide (IIa or IIb), thus demonstrating an unusual *cis*-opening of the epoxide ring.

Although a cardenolide having the 14α,17α-configuration, 3β-hydroxy-5α,14α,17α-card-20(22)-enolide, was synthesized earlier,[1] no cardenolide of this unnatural type possessing 14-hydroxy group has yet been prepared. An attempt[4] to obtain a 14-hydroxy-14α,17α-cardenolide was not successful. Since it was established[5,6] that hydrolytic cleavage of 3β-hydroxy-14α,15α-epoxy-5β-card-20(22)-enolide (14α,17β-cardenolide) resulted in the formation of 3β, 14, 15α-trihydroxy-5β, 14β-card-20(22)-enolide (15α-hydroxydigitoxigenin) (14β, 17β-cardenolide), hydrolytic cleavage of 3β-hydroxy-14β, 15β-epoxy-5β, 17α-card-20(22)-enolide (Ia) (14β,17α-cardenolide) has been performed in anticipation of the possible formation of a 14-hydroxy-14α, 17α-cardenolide. While the formation of the desired cardenolide has not been accomplished in this way, some stereochemically interesting findings were obtained in connection with this work which are described in this paper.

Treatment of Ia[7] in 50% methanol containing 5% hydrochloric acid yielded a mixture of reaction products, whose thin-layer chromatography (TLC) revealed the disappearance of Ia and the formation of three products as shown in Fig. 1a. Chro-

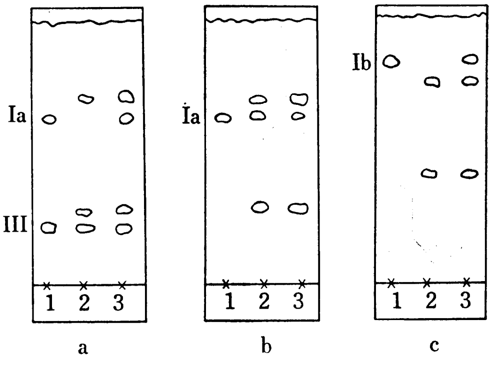

Fig. 1. Thin-Layer Chromatography

a : reaction product obtained after treatment of Ia with HCl in 50% MeOH: 1) Ia+III, 2) reaction product, 3) 1+2
b : reaction product obtained after treatment of Ia with HCl in $CHCl_3$:1) Ia, 2) reaction product, 3) 1+2
c : reaction product obtained after treatment of Ib with HCl in $CHCl_3$:1) Ib, 2) reaction product, 3) 1+2

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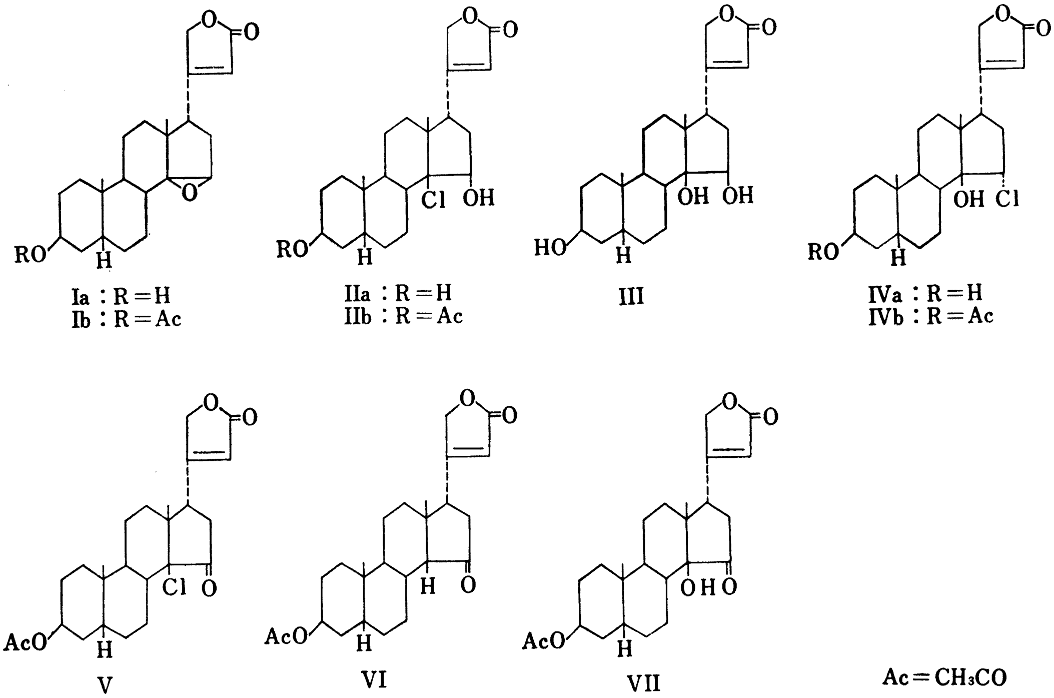
[1] Part III: M. Okada and Y. Saito, *Steroids*, **6**, 645 (1965).
[2] This work was presented at the 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, July, 1970.
[3] Location: *Takada 3-Chome*, *Toshima-ku*, *Tokyo*.
[4] M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **17**, 515 (1969).
[5] M. Okada and M. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965).
[6] H. Ishii, T. Tozyo, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963).
[7] M. Okada, A. Yamada, Y. Saito, and M. Hasunuma, *Chem. Pharm. Bull.* (Tokyo), **14**, 496 (1966). Ia used in this work was prepared from 3β-hydroxy-5β, 17α-card-14, 20(22)-dienolide (β-anhydro-17α-digitoxigenin) by treatment with monoperphthalic acid.[8]
[8] T. Wada and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **13**, 308 (1965).

matographic speparation of the mixture using a column of silica gel afforded three substances Ia, IIa, and III. Isolation of the starting material Ia that could not be detected in the reaction product by TLC was reasonably explained by the conversion of IVa produced in the reaction into Ia during the chromatographic procedure as indicated below. The second product, 3β,15β-dihydroxy-14-chloro-5β,14β,17α-card-20(22)-enolide (IIa), gave a positive Beilstein test and its structure as well as configuration were definitely established on the basis of the experimental evidences which are presented below. The third produst III was shown to be identical with 3β,14,15β-trihydroxy-5β,14β,17α-card-20(22)-enolide by direct comparison with the authentic sample reported earlier.[4]



Ia : R=H
Ib : R=Ac

IIa : R=H
IIb : R=Ac

III

IVa : R=H
IVb : R=Ac

V

VI

VII

Ac=CH$_3$CO

Chart 1

On the other hand, Ia was treated with hydrogen chloride in chloroform, and TLC of the resulting reaction product indicated the presence of IIa and IVa together with Ia (Fig. 1b). Direct crystallization of the product afforded IVa that gave a positve Beilstein test. This compound was found to be labil and did not give a satisfactory result in elemental analysis even after repeated crystallizations. On chromatography of the mother liquor from recrystallization of IVa using a silica gel column, Ia and IIa were obtained.

Treatment of Ib, the acetate of Ia, with hydrogen chloride in chloroform afforded two products, IIb and IVb, after direct crystallization of the resulting reaction product, whose TLC is given in Fig. 1c. These two compounds gave a positive Beilstein test. Satisfactory result in elemental analysis was obtained with IIb, but not with IVb which was also found to be unstable.

In order to clarify the structure and configuration of IIa derivable from IIb by acid hydrolysis IIb was oxidized with chromium trioxide in acetic acid to give a chloroketone (V). Treatment of V with sodium borohydride regenerated IIb. The chloroketone (V) exhibited a positive Cotton effect in its optical rotatory dispersion (ORD) curve as shown in Fig. 2, in accordance with 14-chloro-15-oxo-5β,14β-cardenolide[6] as well as 14-chloro-15-oxo-5β, 14β-etianic acid.[9] As a negative Cotton effect had been observed with 14β-hydroxy-15-oxo-5β, 14β-cardenolide,[6] the corresponding 17α-cardenolide (VII) was prepared for comparison, whose ORD curve is shown in Fig. 2 together with that of V. It exhibited also a negative Cotton effect, thus demonstrating that the steric configuration of the butenolide ring at 17-position does not affect the Cotton effect. Reduction of V with zinc and acetic acid yielded a ketone identical with 3β-acetoxy-15-oxo-5β,14β,17α-card-20(22)-enolide (VI) reported previously.[8] Accordingly, the structure and configuration of IIa and IIb have been established as 3β,15β-dihydroxy-14-chloro-5β,14β,17α-card-20(22)-enolide and its 3-mono-acetate respectively (Chart 1). In connection with this reduction of V it is worth notice that 14-chloro-15-oxo-5β,14β-cardenolide[6] and 14-chloro-15-oxo-5β,14β-etianic acid[9] gave the corresponding dechlorinated compounds possessing 14α-configuration on treatment with zinc and acetic acid.

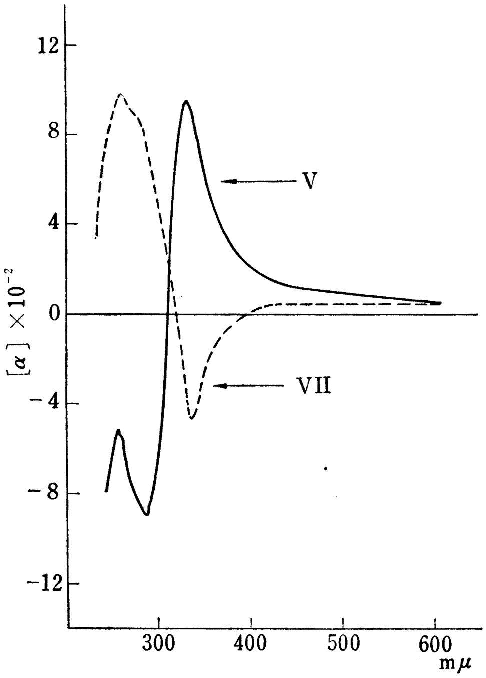


Fig. 2. Optical Rotatory Dispersion Curves of V and VII in Dioxane

The observed unusual *cis*-opening of the β-epoxide ring in Ia or Ib giving β-*cis*-chlorohydrin (IIa or IIb) and β-*cis*-glycol (III) may be explained by the preferential front-side attack of Cl^- or OH^- against the carbonium ion of C-14 owing to the α-configuration of the bulky butenolide ring at 17-position.

The structures of IVa and IVb, on the other hand, could not be established definitely owing to their instability. However, it seemed quite reasonable to give the indicated structures in Chart 1 (3β,14-dihydroxy-15α-chloro-5β,14β,17α-card-20(22)-enolide and its acetate) to them principally based on the following observations: (a) Acetylation of IVa in the usual way with acetic anhydride and pyridine gave IVb and acid hydrolysis of IVb regenerated IVa, though in low yield: (b) Treatments of IVa and IVb with neutral alumina, silica gel or Silica Gel H regenerated Ia and Ib respectively, while IIa and IIb were unchanged. On the basis of essentially similar observations, halohydrins[10,11] derived from Δ^{14}- or 14β,15β-epoxy-steroids were formulated as 14β-hydroxy-15α-halo-steroids.

Experimental[12]

Treatment of 3β-Hydroxy-14β, 15β-epoxy-5β, 17α-card-20(22)-enolide (Ia) with Hydrochloric Acid in Methanol——A solution of Ia (94 mg) in a mixture of MeOH (100 ml) and 10% HCl (100 ml) was allowed

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[9] A. Lardon, H.P. Sigg, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1457 (1959).

[10] M. Heller, F.J. McEvoy, and S. Bernstein, *Steroids*, **3**, 193 (1964).

[11] Ch.R. Engel and G. Bach, *Steroids*, **3**, 593 (1964).

[12] Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (UV) spectra were measured in 99% EtOH solution. Infrared (IR) spectra were determined in KBr disks on Hitachi EPI-S2 spectrophotometer; sh=shoulder. TLC plate was prepared according to the Stahl's procedure using Silica Gel H (E. Merck AG) as adsorbent unless otherwise noticed. The solvent system used was methyl ethyl ketone–heptane (1:1), and the cardenolide spots were revealed by heating plate at 110° for 10 min after spraying 95% H_2SO_4 or Kedde reagent.

to stand for 18 hr at 20°. The solution was neutralized with 5% Na_2CO_3, and MeOH was removed under reduced pressure. After the addition of $CHCl_3$ to the reaction mixture, the organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crystalline residue (114 mg), whose TLC is shown in Fig. la. The residue was chromatographed on a column of silica gel (12 g, E. Merck AG) by successive elution with benzene, benzene–EtOAc (10:1, 7:1, 4:1, 2:1, 1:1), EtOAc, and Et-OAc–MeOH (5:1, 1:1). The fraction (20 mg) eluted with benzene–EtOAc (2:1, 1:1) was recrystallized from MeOH to give Ia (10 mg), mp 220—230°. The fraction (30 mg) eluted with benzene–EtOAc (1:1) was recrystallized from $CHCl_3$–MeOH to afford 20 mg of IIa. mp 210—214°. $[\alpha]^{26}_{D}-8.1°$ ($c=0.41$, $CHCl_3$). UV λ_{max} $m\mu$ (log ε): 216 (4.18). IR ν_{max} cm^{-1}: 3460 (OH), 1820, 1730, 1720, 1628 (butenolide). *Anal.* Calcd. for $C_{23}H_{33}O_4Cl$: C, 67.55; H, 8.13. Found: C, 67.73; H, 8.00.

Further elution with benzene–EtOAc (1:1), EtOAc, and EtOAc–MeOH (5:1, 1:1) gave a crystalline mixture (45 mg) of IIa and III, which were separated by repeated fractional crystallizations from MeOH to afford III (25 mg), mp 228—231°, and IIa (5 mg), mp 209—213°. The former was identical with an authentic sample of III[4] in TLC, mixed melting point and comparison of the IR spectrum.

Treatment of 3β-Hydroxy-14β, 15β-epoxy-5β, 17α-card-20(22)-enolide (Ia) with Hydrogen Chloride in Chloroform——Into a solution of Ia (85 mg) in $CHCl_3$ (3 ml) was introduced anhydrous hydrogen chloride at 0° for 30 min. The solution was then washed consecutively with H_2O, 5% Na_2CO_3, and H_2O, and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* yielded a crystalline residue (86 mg), whose TLC is shown in Fig. 1b. It was recrystallized from $CHCl_3$–MeOH to afford IVa (28 mg), mp 175—182° (decomp.). IR ν_{max} cm^{-1}: 3440 (OH), 1800, 1732, 1640 (butenolide).

The mother liquor from recrystallization of IVa was evaporated to dryness. The residue (55 mg) was chromatographed on a column of silica gel (6 g) by successive elution with benzene, benzene–EtOAc (10:1, 5:1, 5:2, 1:1), and EtOAc. The fraction eluted with benzene and benzene–EtOAc (10:1, 5:1, 5:2, 1:1) gave Ia (36 mg), mp 220—230°, after recrystallization from MeOH. The fraction eluted with benzene–EtOAc (1:1) and EtOAc was recrystallized from $CHCl_3$–MeOH to afford IIa (19 mg), mp 210—215°.

Treatment of 3β-Acetoxy-14β, 15β-epoxy-5β, 17α-card-20(22)-enolide (Ib) with Hydrogen Chloride in Chloroform——Into a solution of Ib (520 mg) in $CHCl_3$ (6.8 ml) was introduced anhydrous hydrogen chloride at 0° for 45 min. Working up in the same way as described above gave a crystalline residue (560 mg), whose TLC is given in Fig. 1c. Repeated recrystallizations of the residue from $CHCl_3$–MeOH afforded IIb (104 mg), IVb (210 mg), and a mixture (244 mg) of IIb and IVb. IIb; mp 230—235° (decomp.). $[\alpha]^{27}_{D}$ $-4.2°$ ($c=0.95$, $CHCl_3$). UV λ_{max} $m\mu$ (log ε): 216 (4.21). IR ν_{max} cm^{-1}: 3600 (OH), 1807, 1750, 1725, 1635 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{35}O_5Cl$: C, 66.57; H, 7.82. Found: C, 66.58; H, 7.84. IVb; mp 197—204° (decomp.). IR ν_{max} cm^{-1}: 3450 (OH), 1785, 1750, 1735, 1720, 1630 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{35}O_5Cl$: C, 66.57; H, 7.82. Found: C, 65.93; H, 7.81.

Conversions of IVa and IVb into Ia and Ib by Treatments with Al_2O_3 and Silica Gel or Silica Gel H——a) To a solution of IVa (4.6 mg) in a mixture of $CHCl_3$ (0.25 ml) and MeOH (0.25 ml) was added neutral Al_2O_3 (180 mg, activity grade III—IV[13]), and the mixture was allowed to stand at room temperature for 48 hr. After filtration of the Al_2O_3, it was washed with acetone. The combined filtrates were concentrated to dryness to give a crystalline residue (3.6 mg) which was recrystallized from acetone–ether to afford Ia (2 mg), mp 220—232°, identical with an authentic sample in TLC, mixed melting point and comparison of the IR spectrum. Conversion of IVa into Ia by treatment with silica gel or Silica Gel H was also demonstrated in the similar way.

b) Silica gel (200 mg) was added to a solution of IVb (5 mg) in a mixture of $CHCl_3$ (0.3 ml) and MeOH (0.3 ml), and the mixture was allowed to stand for 72 hr at room temperature. After filtration of the silica gel, it was washed with acetone. The combined filtrates were concentrated to dryness to give a crystalline residue (3.5 mg) which was recrystallized from acetone–ether to afford Ib (2 mg), mp 175—184°, identical with an authentic specimen of Ib in TLC, mixed melting point and comparison of the IR spectrum.

3β, 15β-Dihydroxy-14-chloro-5β, 14β, 17α-card-20(22)-enolide(IIa)——A solution of IIb (7.8 mg) in a mixture of MeOH (6 ml), $CHCl_3$ (4 ml), H_2O (1 ml), and 35% HCl (1.5 ml) was allowed to stand at 20° for 20 hr. After addition of H_2O (10 ml) the reaction mixture was concentrated *in vacuo* to precipitate a crystalline product which was extracted with $CHCl_3$. The organic layer was washed with H_2O, and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline residue (6.5 mg), whose TLC indicated the presence of IIa together with IIb. Preparative TLC of the residue on Aluminium Oxide G (E. Merck AG) using methyl ethyl ketone–heptane (1:1) as solvent afforded 3.5 mg of IIa after recrystallization from $CHCl_3$–MeOH, mp 214—217°, identical with the sample of IIa described above in the mixed melting point and comparison of the IR spectrum.

3β, 14-Dihydroxy-15α-chloro-5β, 14β, 17α-card-20(22)-enolide (IVa)——A solution of IVb (30 mg) in MeOH (20 ml) and 10% HCl (10 ml) was allowed to stand at 20° for 20 hr. Working up in the way as described above yielded a crystalline product (25 mg). It was repeatedly recrystallized from $CHCl_3$–MeOH

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13) S. Heřmánek, V. Schwarz, and Z. Cekan, *Collection Czech. Chem. Commun.*, 26, 3170 (1961).

to give IVa (5 mg), mp 175—180° (decomp.), identical with the above sample of IVa in TLC, mixed melting point and comparison of IR spectrum.

Acetylation of this IVa (3 mg) in the usual way with acetic anhydride and pyridine gave IVb (2 mg) whose IR spectrum was identical with that of the authentic specimen in all respects.

3β-Acetoxy-14-chloro-15-oxo-5β, 14β, 17α-card-20(22)-enolide (V)——A solutlon of IIb (110 mg) and CrO_3 (26 mg) in AcOH (7 ml) was allowed to stand at 20° for 30 min. To the reaction mixture was added MeOH (1 ml), and then H_2O (50 ml). The product was extracted with $CHCl_3$, and the organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crystalline residue (115 mg), which was recrystallized from $CHCl_3$–MeOH to afford V (95 mg). mp 241—250° (decomp.). $[\alpha]_D^{24}+83.0$ ($c=0.066$, dioxane). UV λ_{max} mμ (log ε): 214.5 (4.19). IR ν_{max} cm^{-1}: 1790, 1750, 1720, 1635 (butenolide, acetyl and C-15 C=O). *Anal.* Calcd. for $C_{25}H_{33}O_5Cl$: C, 66.88; H, 7.41. Found: C, 66.84, H, 7.47. The ORD curve of V is given in Fig. 2.

Sodium Borohydride Reduction of V——To a solution of V (18 mg) in a mixture of MeOH (40 ml) and dioxane (8 ml) was added $NaBH_4$ (76 mg) at 0° for 10 min, and the reaction mixture was allowed to stand at 18° for 18 hr. After acidification with AcOH the reaction mixture was concentrated *in vacuo* and the product was extracted with $CHCl_3$. The organic layer was washed with H_2O, and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline residue (17.6 mg), which was recrystallized from $CHCl_3$–MeOH to afford IIb (13.5 mg), mp 225—230° (decomp.), identical with the authentic sample in TLC, mixed melting point and comparison of the IR spectrum.

Conversion of V into 3β-Acetoxy-15-oxo-5β, 14β, 17α-card-20(22)-enolide (VI)——A solution of V (52 mg) and $CH_3COONa \cdot 3H_2O$ (49 mg) in a mixture of AcOH (30 ml) and MeOH (30 ml) was refluxed for 1.5 hr with zinc dust (60 mg) while stirring vigorously. After filtration of zinc dust the reaction solution was concentrated *in vacuo* to a small volume. The product was extracted with $CHCl_3$ and the organic layer was washed with H_2O and dried. Evaporation of the solvent gave a crystalline residue (48 mg), which was recrystallized from MeOH to afford VI (29 mg), mp 235—237°, identical with an authentic sample prepared according to Wada, *et al.*[8] in the mixed melting point and comparison of the IR spectrum.

3β-Acetoxy-14-hydroxy-15-oxo-5β, 14β, 17α-card-20(22)-enolide (VII)——A solution of 3β-acetoxy-14, 15β-dihydroxy-5β, 14β, 17α-card-20(22)-enolide[4] (198 mg) in pyridine (2.4 ml) was added to a slurry of chromic anhydride (307 mg) and pyridine (3 ml). The reaction mixture was allowed to stand at room temperature for 20 hr, poured into ice–water, and extracted with ether. The organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent afforded a residue (190 mg), which was chromatographed on a column of neutral alumina (10 g) by successive elution with hexane–benzene (1:1, 1:4), benzene, benzene–EtOAc (5:1, 5:3, 1:1). The fraction (30 mg) eluted with benzene–EtOAc (5:1) was recrystallized from acetone–ether to give VII (20 mg). mp 245—252°. $[\alpha]_D^{24}+16°$ ($c=0.062$, dioxane). UV λ_{max} mμ (log ε): 216 (4.18). IR ν_{max} cm^{-1}: 3500 (OH), 1798, 1748, 1720, 1628 (butenolide, acetyl and C-15 C=O). *Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 70.01; H, 8.11.

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