SYNTHESIS OF 2α- AND 2β-HYDROXYCORTISOL

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Received June 17, 1963

ABSTRACT

The synthesis (in 1 gm. amounts) of 2α-hydroxy- (I) and 2β-hydroxycortisol (II), their 2,21-diacetates (I-diac., II-diac.) and 2,11β,21-triacetates (I-triac., II-triac.) is described. Cortisol 21-acetate was acetoxylated with lead tetraacetate in glacial acetic acid. Following saponification in methanolic potassium hydroxide a mixture of I and II was obtained by partition chromatography in approximately 13% yield. Reacetylation of the purified mixture of I and II with acetic anhydride and pyridine at room temperature and subsequent partition chromatography yielded pure I- and II-diac. and 5-10% of I- and II-triac. I and II were obtained by saponification of I- and II-diac. in methanolic potassium hydroxide without inversion. The structure of the isolated compounds was assigned from their elemental analyses, molecular rotation, NMR, ultraviolet and infrared spectra.

Introduction

The synthesis of 2α-hydroxycortisol (2α,11β,17,21-tetrahydroxypregn-4-ene-3,20-dione) (I) (albeit in poor yield) and its identification as one of the $C_{21}O_6$ corticosteroids excreted in guinea pig urine ("steroid IIa" obtained following cortisol feeding[1]) has been previously described.[2] This constituted the first demonstration of the occurrence of C-2 hydroxylation in an animal. The identity of steroid IIa with I was confirmed independently by Péron and Dorfman[3] by oxidation of the 2α-hydroxycortisol 2,21-diacetate (I-diac.) to the known 2α-hydroxycortisone 2,21-diacetate.[4]

2α-Hydroxycortisol has also been isolated from the urine of untreated guinea pigs[5] and found in elevated concentrations in the urine of strain 13 and 2 guinea pigs with leukemia and liposarcoma[6]. This steroid was found

in lower concentrations in Hartley guinea pigs[5] than in strain 2 or 13 animals[6] indicating the possibility of strain differences in the excretion of this steroid. Nadel, Young and Hilgar[7] in a further study of groups of guinea pigs of these types confirmed the earlier observations. Utilizing a recently available method which allows the study of the excretion of corticosteroids in individual animals[8] even a more striking difference between animals was noted.[9]

The origin or the site of 2α-hydroxycortisol biosynthesis in the guinea pig has not been definitively determined. Axelrod, Miller and Herling[10] have demonstrated 2β-hydroxylation of testosterone in a dog liver perfusion. Although I has been isolated from guinea pig urine following non-labelled cortisol feeding[1], experiments with labelled cortisol have been hampered by the unavailability of larger quantities of I for isotope dilution studies, while attempts to demonstrate 2α-hydroxylation of cortisol by surviving guinea pig liver or adrenal tissue with unlabelled cortisol have been thus far unsuccessful (unpublished results). The possibility that 2α-hydroxycortisol is actually an artifact arising from the primary formation of 2β-hydroxycortisol (2β,11β,17,21-tetrahydroxypregn-4-ene-3,20-dione) (II) merits consideration but again insufficient I has been available for investigation of this reaction path.

This publication describes the synthesis of 2α-hydroxycortisol and its diacetate and the hitherto undescribed 2β-hydroxycortisol, and its 2,21-diacetate (II-diac.) (in ca 1 gm. quantities) as well as the 2,11β,21-triacetates of I (I-triac.) and II (II-triac.).

Results and Discussion

The reaction of cortisol 21-acetate with lead tetraacetate, from which prednisolone 21-acetate was previously isolated[11], yielded also I- and II-diac., which, however, could not be efficiently separated directly from the

unreacted cortisol 21-acetate and the other products. The 2α-hydroxycortisol was isolated previously[2] (in poor yield) from such a mixture following saponification with potassium bicarbonate at elevated temperatures[12] followed by chromatography. Saponification at room temperature yielded a mixture of I and II from which II was obtained in a pure state by crystallization but I could not be isolated in amounts more than a few milligrams. The identity of II was surmised from its spectrum in alkali[13], chromatographic mobility and infrared spectrum. While this phase of this study was in progress Rao and Axelrod[14] reported that saponification with potassium hydroxide in methanol of 2β-hydroxytestosterone 2,17-diacetate did not cause an inversion of the unstable 2β-hydroxy group and gave 2β-hydroxytestosterone 17-acetate in good yield. Since the yield of saponification of the total acetoxylation mixture with potassium bicarbonate was not satisfactory, the saponification procedure of Rao and Axelrod[14] was utilized and led in 13% yield to a mixture of the 2α- and 2β-hydroxycortisols. However, the separation of the free compounds by partition chromatography was difficult because of identical mobilities in many systems. In the two partition systems which afforded a slight separation, I and II could be isolated pure only in mg. amounts because of the relatively low capacity of the systems. The separation of the diacetates obtained by acetylation of the mixture I and II with acetic anhydride and pyridine at room temperature appeared to be more promising and a system containing benzene, skellysolve C, methanol and water was developed which allowed the clean separation of I- and II-diac. During the acetylation two less polar by-products were formed in 5-10% yields. These were identified as the 2,11β,21-triacetates.

The purified I- and II-diacetates were saponified in methanolic potassium hydroxide to give the respective free compounds in almost quantitative yield.

The proof of structure of the isolated I and II and their di- and triacetates rests on the following evidence. All these steroids had the correct

elemental analyses for $C_{21}O_6$ compounds (and their respective acetates). The ultraviolet spectra in methanol of these steroids showed maxima in the range of 241-245 mμ with molecular extinction coefficients from 13,900 to 16,600, which is consistent with the presence of a Δ^4-3-keto system. Their spectra in alkali exhibited the unique characteristics of 2-hydroxy-Δ^4-3-ketones described by Meyer.[13] The infrared spectra* showed the expected functional region bands. I-diac. exhibited the vicinal effect of the 2α-acetoxy group by a shift of the C-3 conjugated carbonyl to 1696 cm^{-1}. In II-diac, on the other hand, the 3 conjugated carbonyl absorbed at 1673 cm^{-1}. The I- and II-triac. both showed a vicinal effect (conjugated C-3 carbonyl absorbed at 1687 cm^{-1} in both KBr pellet and in chloroform solution). The infrared spectrum of I-diac. was identical in all respects to the 2α-hydroxycortisol 2,21-diacetate isolated from pools of guinea pig urine described previously.[2] I obtained in this study exhibited a higher melting point and had a better resolved infrared spectrum than the synthetic 2α-hydroxycortisol described earlier which was not an analytical specimen.[2] The molecular rotations of the compounds described and the calculated contributions of the 2α and 2β-hydroxy and acetoxy groups are summarized in Table I. The molecular rotation contribution of the 2α-hydroxyl (+79) is within the range found by Rosenkranz et al.[4] (+51 to +90). The strong levorotatory contribution of the 2β-hydroxyl (-771) is also within the range found and quoted by Rao et al.[15] and Smith et al.[16] (-519 to -768). The small contributions of the 2α-acetoxy groups (+25 and +56) was higher than that reported by Rosenkranz et al.[4] (-10 to -74) but this may be due to the effect of solvent, since the comparisons were not all made from data obtained in the same solvents and with small rotations this may have a large effect. The contribution of the 2β-acetoxy groups (-500 to -443) is within the range of values which can be calculated from the data of Rao et al.[15] and Sondheimer et al.[12] (-544 to

Table I

Molecular Rotation Data of 2α- and 2β-Hydroxycortisol and their Respective 2,21-Diacetates and 2,11β,21-Triacetates

	M_D					Contribution			
		2α-OH	2α-OAc	2β-OH	2β-OAc	2α-OH	2α-OAc	2β-OH	2β-OAc
Cortisol	+604[a]	+683[d]		-167[d]		+79[d]		-771[d]	
Cortisol-21-acetate	+637[b]		+662[d]		+137[d]		+25[d]		-500[d]
Cortisol-11β,21-diacetate	+746[c]		+802[e]		+303[e]		+56[e]		-443[e]

[a] From N. L. Wendler, R. P. Graber, R. E. Jones, and M. Tishler, $[\alpha]_D^{25}$ + 167° (J. Am. Chem. Soc. 74, 3630 (1952)) (EtOH)

[b] From ibid. $[\alpha]_D^{25}$ + 157.5° (dioxane)

[c] From E. P. Oliveto, C. Gerald, and E. B. Hershberg, $[\alpha]_D^{25}$ + 167.1° ($CHCl_3$) (Arch. Biochem. Biophys. 43, 234 (1953))

[d] In Dioxane

[e] In Chloroform

Table II

Molecular Rotation Differences Between 2α and 2β
Hydroxy and Acetoxy Δ4-3-Ketones

	ΔM_D (2α - 2β)
2-hydroxycortisol	850[a]
2-hydroxyandrost-4-ene-3,17-dione	712[b]
2-hydroxyprogesterone	783[b]
2-hydroxycortisol 2,21-diacetate	525[a]
2-hydroxycortisol 2,11β,21-triacetate	499[a]
2-hydroxyandrost-4-ene-3,17-dione acetate	534[b]
2-hydroxytestosterone 2,17-diacetate	528[c]
2-hydroxyprogesterone acetate	496[b]

a - This study
b - Rao and Axelrod[14]
c - Sondheimer et al.[12]

-486). The difference in molecular rotation ΔM_D (2α-2β) between the respective 2α- and 2β-hydroxy and acetoxy isomers and of some published pairs are summarized in Table II. It is evident from this table that the values of the compounds reported here are in agreement with those from the literature. The difference in the ΔM_D between the 2-hydroxy and 2-acetoxy derivatives is noteworthy. Further evidence for the assigned structure was obtained from the optical rotatory dispersion of the compounds described here in comparison with 2α- and 2β-hydroxy and acetoxy steroids described by Rao et al.[15] (to whom thanks are due for providing samples). These data which will be presented in a forthcoming publication[17], conclusively corroborate the configurational assignments given here. The NMR spectrum*** of I-diac. exhibited the C-2 proton as a quartet with peaks centered at 328, 320, 315 and 310 cps. Coupling constants derived on the basis of a first order approximation are $J_{2\beta,1\alpha}$ = 11.5 cps and $J_{2\beta,1\beta}$ = 6.7 cps. Dihedral angles were calculated by the Karplus equation with parameters as modified by Williamson and Johnson[18]: $\theta_{2\beta,1\beta}$ =

27-35° and $\theta_{2\beta,1\alpha}$ = 147-155°. These constants are consistent with the assigned structure and indicate a normal ring A half-chair conformation with moderate distortion for the 2α-acetoxy isomer. The corresponding coupling constants of I-triac. were $J_{2\beta,1\alpha}$ = 13.8 cps and $J_{2\beta,1\beta}$ = 6.3 cps with dihedral angles $\theta_{2\beta,1\alpha}$ = 157-158° and $\theta_{2\beta,1\beta}$ = 37-38° which again is consistent with the given assignment. II-diac. exhibited a C-2 quartet with peaks centered at 329, 325, 316 and 311 cps. The coupling constants were $J_{2\alpha,1\beta}$ = 13.7 cps and $J_{2\alpha,1\alpha}$ = 4.7 cps and the calculated dihedral angles were $\theta_{2\alpha,1\alpha}$ = 38-47° and $\theta_{2\alpha,1\beta}$ = 158-167°. These values which are not consistent with a normal half-chair conformation for a ring A unsubstituted Δ^4-3-keto steroids do fit a "twist" but not a boat conformation and largely parallel the findings of Williamson and Johnson[18] in the case of 2β-acetoxy-5α-3-keto steroids. The II-triac. exhibited coupling constants $J_{2\beta,1\beta}$ = 13.2 cps and $J_{2\beta,1\alpha}$ = 5.5 cps with dihedral angles $\theta_{2\alpha,1\alpha}$ = 35-42° and $\theta_{2\alpha,1\beta}$ = 155-162° again indicating a ring A in "twist" conformation.

The location of the third acetate group at 11β in I- and II-triac. is based on the failure of the compounds to undergo oxidation with chromic acid and on the NMR spectral data. Thus the 11α-proton which in I-diac. was centered at 5.60 τ (264 cps) moved to 4.56 τ (326 cps) in I-triac. The corresponding τ values of the 11α proton for the 2β-acetoxy derivatives were 5.39 (276 cps) and 4.46 (333 cps). The position of the 11α proton in I-diac. and II-diac. was consistent with that of other 11β-hydroxy compounds while the shift downfield following acetylation was in the proper direction and of the correct magnitude noted for the conversion of many hydroxylated substances to their acetate esters. The isolation of the 11β-acetoxy compounds is in line with the results of Kemp et al.[19] who also showed that some acetylation of the hindered 11β-hydroxy group occurs in acetic anhydride-pyridine at

room temperature in the absence of acid.

Experimental

Melting points were determined on a micro hot stage (H. Bock, Frankfurt/M) and are corrected. The ultraviolet and absorption spectra were determined with the Cary model 14 spectrophotometer. Infrared spectra were recorded in KBr with a Beckman IR7 spectrophotometer.

Preparative Partition Chromatography. The partition chromatography of the larger quantities (0.5 gm or more) was done on 5.1 x 115 cm. columns equipped with sintered glass disks at the bottom. The columns were packed dry with celite 545 (800 gm) containing 0.5 ml/gm of stationary phase. The packing was done essentially as described by Kelly, Bandi, Shoolery and Lieberman.[20] The mobile phase was then carefully introduced to the top of the column so as not to disturb the top layer. It was observed that the uniformity of the uppermost layer was very important for good resolution. An imperfect upper layer (due to too fast mobile phase delivery and air bubble formation) caused considerable spreading and merging of zones. The holdback volume of the column was checked by running through sudan III dissolved in a small volume (10-20 ml) of mobile phase. A phenomenon that was observed in all of the columns was the distortion of the moving zone into a funnel shape which became more exaggerated the longer the column. This could not be observed while the zone was in the body of the column but only as the dye began to emerge from the bottom of the column: the zone would always appear first in the center of the column. This, of course, caused some spreading and resulted probably from the celite being more densely packed near the wall of the column than in the center. This phenomenon was lessened to some extent when thinner layers were used but was never eliminated entirely. Despite this it seems that the resolution was as good if not better than that obtained with columns packed with mobile phase present with a Martin packer.

Acetoxylation of Cortisol Acetate with Lead Tetraacetate. Cortisol acetate (20 gm) was dissolved by heating in 400 ml glacial acetic acid containing 5 ml acetic anhydride. Lead tetraacetate (30 gm) was added, nitrogen passed through the solution, and the inside temperature was kept at 85°C for approximately 2 hours or until KI-starch paper indicated that all of the lead tetraacetate was consumed. The cooled reaction mixture was taken up into approximately 5 liters of methylene chloride, shaken with sufficient concentrated sodium hydroxide to neutralize 90% of the acetic acid, washed with sodium bicarbonate and water and evaporated to dryness.

Saponification and Partition Chromatography of the Reaction Mixture. The reaction mixture residue (ca. 23 gm) was dissolved in 1,600 ml of freshly redistilled methanol which had been previously boiled while nitrogen was passed through the solution. To the solution 60 ml of 1 M methanolic potassium hydroxide (prepared from the same methanol) was added, thoroughly mixed, and left at 30° under nitrogen for 10 minutes. Then 6 ml of water (oxygen free) was added and the solution kept for an additional 2 minutes. The solution was made slightly acid with 1 M acetic acid, the methanol evaporated under reduced pressure, and extracted with ca. 5 liters of ethyl acetate. The ethyl acetate was washed with saturated sodium bicarbonate and water and evaporated under reduced pressure. The saponified mixture (17-18 gm) was chromatographed in two portions in the partition system: ethylene chloride 80, methanol 6.8, and water 13.2 volumes %, the equilibrated lower phase being used as the mobile phase. Each portion was delivered to the column after solution in 40 ml of mobile phase and additional small amounts of methanol as required to completely dissolve the mixture. Fifty ml fractions were collected and the holdback volume of the column was 25 fractions. The mixture of I and II emerged from the column in fractions 55-95.

Attempts to Separate I and II. Several partition systems were tried to effect separation of the 2α- and 2β-hydroxycortisols. On paper, two systems were found which gave a separation when run for long periods on 115 cm. long papers. These were a) the ethylene chloride system described previously[8] and b) system E_2B of Eberlein and Bongiovanni.[21] Four mg of the mixture was chromatographed on four 15 cm. wide 115 cm. long Whatman No. 2 papers and chromatographed for 42 hours in the ethylene chloride system. Two distinct zones located 82 and 93 cm. from the starting line were visible under ultraviolet light. Elution of these zones and further purification by short partition chromatography on celite yielded the respective isomers which were identified conclusively by their infrared spectra. II ran slower in this system than I. Column partition chromatography of 50 mg of the mixture in this system, however, did not result in a separation of the 2 isomers even on a 2 × 130 cm. column. Paper chromatography in the E_2B system was difficult because of very slow solvent front running rates. In 27 hours the faster zone (in this case II) moved 28 cm. The R_f values (probably inaccurate because of solvent evaporation from the strips) were 0.42 and 0.45. However, column chromatography in this system again did not result in a clean separation. There was considerable spreading possibly because of the low capacity. From the early and last fractions some pure II and I were isolated. The system, however, was unsuitable for the chromatography of larger quantities and it also was found that the stationary phase began to wash off the column in cloudy droplets several hours after the chromatography was started. A modification of the E_2B system (devised in order to increase the capacity) which consisted of isooctane 60:ethyl acetate 15:t. butanol 17:methanol 8: water 12.5 vols. also afforded a separation on paper but was unsuitable as a preparative method. The systems described by Frantz et al.[22] (system Y

and Z) as well as the systems reported by Fukushima et al.[23] failed to separate these isomers.

Preparative Chromatographic Separation of I- and II-diac. and I- and II-triac. Several systems of compositions similar to the B_3 system described by Bush[24] were tried. The following was found to give a good separation of I- and II-diac.: benzene 5:skellysolve C 5:methanol 4:water 1 (vols.). The R_f values in this system (30°) in 60 cm. tanks were 0.43 (I-diac.) and 0.53 (II-diac.). This system had a satisfactory capacity and was used for the preparative separation of I and II-diac. obtained by reacetylation of part of I and II from the preparative ethylene chloride chromatogram described above. The I and II mixture was acetylated with 4 ml acetic anhydride in 5 ml pyridine at room temperature overnight and the solvents removed in vacuo with minimal heating. The acetylated mixture (ca. 3.0 gm. was used) was delivered to the column dissolved in 60 ml mobile phase containing ca. 3 ml methanol. Fifty ml fractions were collected (holdback volume of the column was 25 fractions). Three major fractions were obtained: 131-232 (A, 1.0 gm), 72-130 (B, 1.7 gm) and 40-71 (C, 0.3 gm). Fraction A was rechromatographed in the same system and yielded 882 mg of chromatographically homogeneous (by paper) I-diac. Fraction B following rechromatography yielded 1.2 gm of chromatographically (by paper) homogeneous II-diac. Fraction C contained a mixture of I- and II-triac. which do not separate in this system. A suitable system to separate the triacetates was benzene 15: skellysolve C 35:methanol 40:water 10 (vols.). The R_f values on paper at 30° in 60 cm. tall tanks were 0.22 (I-triac.) and 0.28 (II-triac.). When chromatographed in this system on the preparative column and 50 ml fractions were collected, I-triac. emerged in fraction 315-400 (C-1) and II-triac. in 220-295 (C-2).

2α-Hydroxycortisol 2,21-diacetate (I-diac.). A part of I-diac. obtained

from the preparative partition chromatogram was chromatographed on silica gel (10 gm) with methylene chloride-ethanol. The material was eluted with 2-3% absolute ethanol in methylene chloride. Crystallization from benzene-hexane-methanol-water and repeated crystallizations from methanol yielded an analytical sample (prisms) m.p. 238-240°, $[\alpha]_D^{27}$ + 143.0° (dioxane), $\lambda_{max.}$ 241 mμ (ε 15,970), infrared spectrum: 3485 (OH), 2950 (CH), 1740-1745 (acetate C=O), 1710 (20 C=O), 1696 (3 C=O), 1617 (C=C), and 1213-1235 cm^{-1} (acetate). I-diac. gave a peach colored solution with a green fluorescence in sulfuric acid.

Calc. for $C_{25}H_{34}O_8$ (462.6): C, 64.92; H, 7.41

Found: C, 64.84; H, 7.45

2β-Hydroxycortisol 2,21-diacetate (II-diac.). A part of II-diac. from the preparative chromatogram was crystallized first from hexane-benzene-methanol-water and then from methanol. This yielded material melting at 197-99°. When this material was dissolved in methylene chloride and then crystallized from methanol, solvated material was obtained melting unsharply around 120°C. This material exhibited in the infrared the same functional region bands as the previous crop but there were significant differences in the fingerprint region. It was possible to free this material of solvent at 135° under reduced pressure. From methanol containing a little water this solvated material gave another substance melting at 197-199° after becoming opaque at around 120° and exhibiting a characteristic fingerprint region significantly different from the other two spectra although corresponding in the major bands. The thoroughly dried material (sent for elemental analysis) gave still another characteristic fingerprint region spectrum. Although it was possible to interconvert these solvates it was not possible to reproduce their infrared fingerprint precisely. The exact nature of these

solvates was not further studied. The analytical sample had a m.p. 197-99°, $[\alpha]_D^{28}$ + 29.6° (dioxane), $\lambda_{max.}$ 244 mμ (ε 15,280), infrared spectrum: 3535 and 3365-3470 (OH), 2935 (CH), 1745 (acetate C=O), 1720-24 (20 C=O), 1673 (3 C=O), 1612 (C=C) and 1212-1260 cm^{-1} (acetate). In concentrated sulfuric acid a pink to red color (depending on the concentration) with no visible green fluorescence (at low concentration) was obtained.

Calc. for $C_{25}H_{34}O_8$ (462.6): C, 64.92; H, 7.41

Found: C, 64.52; H, 7.23

2α-Hydroxycortisol (I). The combined fractions of I-diac. from the preparative chromatogram were hydrolyzed as described under "saponification of the acetoxylation reaction product." Following the evaporation of the solvent, water was added, the crystals collected on a Buchner funnel, washed with water and air dried. Crystallization once from methanol containing a little water afforded rhombohedral (hexagonal) crystals, melting at 213-15°. Further crystallizations from acetone-hexane gave small rod shaped crystals. Analytical sample m.p. 229-33°, $[\alpha]_D^{26}$ + 181° (dioxane), $\lambda_{max.}$ 243 (ε 14,280), infrared spectrum: 3540, 3470, 3400 (OH), 2940, 2910, 2870 (CH), 1707 (20 C=O), 1664 (3 C=O), 1612 cm^{-1} (C=C). In sulfuric acid a peach colored solution with a green fluorescence was obtained.

Calc. for $C_{21}H_{30}O_6$ (378.5): C, 66.65; H, 7.99

Found: C, 66.70; H, 7.75

2β-Hydroxycortisol (II). This steroid was isolated following saponification of the combined II-diac. fractions from the preparative chromatogram. Crystallization from methanol containing water and acetone-hexane yielded fine needles. The analytical sample - m.p. 231-34°, $[\alpha]_D^{27}$ -44° (dioxane), $\lambda_{max.}$ 245 mμ (ε 13,900), infrared spectrum: 3510, 3380, 3300 (OH), 2950, 2930 (CH), 1712 (20 C=O), 1680 (3 C=O), 1615 cm^{-1} (C=C). In sulfuric acid II

Table III Spectra in Sulfuric Acid

Steroid	Major Peaks Immediately upon dissolving		2 Hours after dissolving	
	mμ	ε	mμ	ε
2α-Hydroxycortisol-2,21-diacetate	238	17,340	240	19,470
	290	19,400	285	14,590
	382	4,700	337	10,240
	500	13,140	390	8,400
			476	8,670
			505 (shoulder)	8,090
2β-Hydroxycortisol-2,21-diacetate	238	17,160	239	20,580
	287	13,880	280 (shoulder)	14,570
	382	12,000	338	13,040
	498	11,490	388	10,640
			473	10,060
			504 (shoulder)	8,670
2α-Hydroxycortisol	238	13,295	239	10,370
	291	16,990	286	12,690
	381	5,720	335	7,670
	500	8,670	390	7,540
			472	6,970
			497 (shoulder)	6,250
2β-Hydroxycortisol	238	11,360	239	13,920
	293	12,950	277 (shoulder)	10,980
	380	8,770	380 (shoulder)	8,510
	505	6,790	475	6,380
			500 (shoulder)	5,420
2α-Hydroxycortisol-2,11β,21-triacetate	238	11,340	237	14,650
	288	16,080	277	14,020
	379	18,630	382	15,700
	494	1,070	460 (shoulder)	4,300
			531 (shoulder)	2,700
2β-Hydroxycortisol-2,11β,21-triacetate	232	11,120	238	15,700
	284	13,420	262	14,230
	378	18,160	381	15,880
	465	2,230	460 (shoulder)	5,300
			530 (shoulder)	3,040

behaved in the same manner as II-diac.

Calc. for $C_{21}H_{30}O_6$ (378.5): C, 66.65; H, 7.99

Found: C, 66.71; H, 7.93

2α-Hydroxycortisol 2,11β,21-triacetate (I-triac.). This substance was obtained from fraction C-1 by crystallization. It was very soluble in most solvents. Upon standing in acetone-hexane needles were obtained (m.p. 151-157°). Recrystallization from isooctane-acetone afforded long needles (m.p. 161-164°) with some tiny needles melting at 137-142° (probably solvated). Further crystallization from isooctane-acetone afforded an analytical sample, m.p. 162-168° $[\alpha]_D^{28}$+ 159° (chloroform), $\lambda_{max.}$ 240 mμ (ε 16,130), infrared spectrum: 3520 (OH), 2930 (CH), 1744 (acetate C=O), 1728 (20 C=O), 1687 (3 C=O), 1612 (C=C), and 1229-1243 cm^{-1} (acetate). In sulfuric acid a yellow (amber) color was obtained with no visible fluorescence.

Calc. for $C_{27}H_{36}O_9$ (504.6): C, 64.27; H, 7.19

Found: C, 64.16; H, 7.30

2β-Hydroxycortisol 2,11β,21-triacetate (II-triac.). This substance was obtained by crystallizing fraction C-2 described above. Crystallizations from methanol and benzene containing a small amount of acetone gave an analytical sample (needles) - m.p. 251-254°, $[\alpha]_D^{28}$+ 60° (chloroform), $\lambda_{max.}$ 243 mμ (ε 16,600, infrared spectrum: 3540 (OH), 2950 (CH), 1740 (acetate C=O), 1722 (20 C=O), 1687 (3 C=O), 1621 (C=C), and 1243, 1228 and 1213 cm^{-1} (acetate). In sulfuric acid a yellow color was obtained with no visible fluorescence.

Calc. for $C_{27}H_{36}O_9$ (504.6): C, 64.27; H, 7.19

Found: C, 64.38; H, 7.22

All the compounds studied exhibited spectra in alkali which were consistent with those found by Meyer[13] for 2-hydroxy-Δ^4-3-ketones. The sulfuric acid spectra of the described compounds are given in Table III.

Acknowledgment

This work was supported by Research Grant No. CA-05466 from the National Cancer Institute, U. S. Public Health Service, and Research Grant No. P-293A from the American Cancer Society.

Footnotes

*Thanks are due to Neville Bacon for recording and discussing the infrared spectra.

**We wish to thank Thomas A. Wittstruck for determining and interpreting the NMR spectra and for calculating the dihedral angles. The NMR spectra were determined in $CDCl_3$ solution with tetramethylsilane as internal reference and calibrated using the side-band technique. The instrument used was a Varian 4300 n.m.r. spectrometer operated at a frequency of 60 M.C.P.S.

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