BILE ACIDS. LXXIX. SYNTHESIS AND REDUCTION OF 1,4-DIEN-3-ONES OF VARIOUS BILE ACIDS

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ABSTRACT

1,4-Dien-3-ones of various bile acids (IIa-d), their methyl esters (IIe-h), and their formylated derivatives (IIi-k) were synthesized and their reduction investigated by both catalytic and chemical methods as an alternative route to the synthesis of allo bile acids. Lithium-ammonia reduction proved to be the better method for the reduction of these 1,4-dien-3-ones producing the 3-keto- and 3β-hydroxy-allo bile acids (Vb-d) and (VIb-d) in 66-72% yields.

INTRODUCTION

In continuation of studies with 5α- or allo bile acids in this laboratory, improved methods of synthesis have been investigated. A number of methods have been reported for synthesis of allo bile acids (1,2), but these usually involve lengthy separation and/or provide poor yields. In this report studies of the formation and reduction of 1,4-dien-3-ones of various bile acids starting from the readily available 3-oxo-5β-cholan-24-oic acids, its analogs substituted at C-7 and/or C-12, and their methyl esters (I) were undertaken as alternative means for the synthesis of allo bile acids.

MATERIALS AND METHODS

Melting points were determined with a Fisher-Jones melting point apparatus and were uncorrected. TLC was carried out on aluminum sheets pre-coated with silica gel 60 F254 (E. Merck, supplied by VWR Scientific, Chicago, IL) using acetone-hexane (1:1) or acetone-hexane-acetic acid (20:20:1). A 10% solution of phosphomolybdic acid in ethanol was used as spraying agent for developing the plates. Preparative separations were carried out with 20 x 20 cm plates coated with 2 cm silica gel (supplied by Analtech, Newark, DE). Ultraviolet spectra were recorded on a Beckmann DU-7 spectrophotometer. GLC analyses were carried out with a Hewlett Packard 5890A instrument, using 3% OV-1 or OV-17 on packed columns; other conditions were those described earlier (3). Mass spectra were recorded on an LKB 9000 GC-MS instrument. HPLC analyses were done with a Waters HPLC system (4) using an RCM C-18 column (8 mm x 10 cm, 5μ particles) with an RI detector. Lithocholic acid was obtained from Sigma Chemical Co., St. Louis, MO, while the other bile acids were available from stock in this laboratory. All acids were recrystallized before use. Tris (triphenylphosphine)-rhodium chloride, DDQ, K-Selectride, and Silica gel (Davisil, 100-200 mesh) were purchased from Aldrich Chemical Co., Milwaukee, WI. Dioxane was purified by refluxing under nitrogen with 10 wt.% conc. HCl for 3 h followed by separation of the phases, drying of the organic layer over solid KOH, and distillation. The product was then redistilled over $LiAlH_4$ just prior to use. THF was distilled from $LiAlH_4$ before use. All other solvents were analytical or HPLC grade.

Preparation of methyl 3-oxo-chola-1,4-dien-24-oates (IIa-IIk)

Solutions made of 10 mmol each of the 3-keto bile acids (Ia-Id) or the methyl esters (Ie-Ih), or the formylated derivatives (Ii-Ik) (5) in 50 mL of peroxide-free dry dioxane were refluxed with 22 mmol of DDQ for 4-10 h during which the reaction was monitored by TLC. The solution was cooled to room temperature, the precipitated hydroquinone was filtered off, and then washed with 2 x 20 mL of ethyl acetate. The combined organic fractions were then evaporated and the residue was chromatographed over silica gel (100-200 mesh) using acetone/hexane (1:1, 1:2 or 1:3) depending on the polarity of the starting material used. Fractions of the 3-oxo-5β-chola-1-en-24-oic acid derivatives (III), which elute first followed by fractions containing 3-oxo-5β-chola-1,4-dien-24-acid derivatives (IV), were collected and the solvent was evaporated. A second purification using the same conditions was nearly always necessary. The material so obtained was then recrystallized from ethyl acetate/hexane or acetone/hexane after treatment with active charcoal for decolorization.

Table 1 lists those compounds prepared according to this method and their physical data.

Hydrogenation of 1,4-dien-3-ones (II)

Each of the compounds (IIe-IIh,100 mg) was dissolved in 25 mL of ethyl acetate in a suitable flask and was hydrogenated at room temperature and 1 atm. pressure for 4 h after addition of 10-15 mg of 5% Pd on charcoal. The catalyst was filtered off and an aliquot of the mixture was analyzed by GC. The components of the mixture were separated by preparative TLC using acetone/hexane (1:1) for developing the plates. After recording their MS, each of the components was oxidized by refluxing with an excess of Ag_2CO_3/Celite in toluene (6). GC and MS data of the products obtained indicated that in each case a mixture of 5β- and 5α-isomers had been produced which were then separated by HPLC using acetonitrile/water (75:25, 85:15 or 95:5 depending on the polarity of the bile acid) and identified by comparison of their physical constants with those of authentic materials. The results are summarized in Table 1. Identical results were obtained when PtO_2 was used as catalyst.

Hydrogenation of 1,4-dien-3-ones to 4-en-3-ones (IV)

Tris (triphenylphosphine)-rhodium chloride was used as the homogeneous catalyst to hydrogenate selectively the 1,4-dien-3-ones (IIe-IIh). The method was similar to that described (7); 6-8 h of stirring was required for the conversions. After removing the solvent in vacuum the residue was refluxed with acetone/hexane (1:2) (2 x 50 mL) and filtered. The combined organic fractions were evaporated and the product in each case was recrystallized from acetone/hexane.

Reduction of 1,4-dien-3-ones (II) to 3ε-hydroxy-4-enoates (V)

To 50 mg of each of the 1,4-dien-3-ones (IIe-h) in 5 mL of methanol was added a solution of 50 mg $NaBH_4$ in 5 mL methanol. As the mixture was stirred at room temperature, small aliquots were removed periodically for assay by TLC. Although small amounts of starting material were detected after 2 h, each reaction mixture was diluted with water to turbidity and extracted with 2 x 25 mL of ethyl acetate; the extract was washed with saturated NaCl solution, dried over $MgSO_4$, and the solvent was evaporated. Besides the starting material only one other substance was present which gave a characteristic greenish-bluish color with the developing agent. The mixture was separated by preparative layer chromatography to give 75-85% of compounds (Ve-h).

TABLE 1. PHYSICAL DATA OF VARIOUS 1,4-DIEN-3-ONES (IIa-k)

Compound	Reaction Time	Yield %	m.p. °C	λmax(ε)	Mass Spectral Data
IIa	8	23	224-226	245(15,000)	370(M^+); 355(M-CH_3); 269(M-SC); 122(100%)
IIb	6	28	235-238	246(14,500)	386(M^+); 368(M-H_2O); 267 M-(H_2O+SC);122(100%)
IIc	6	23	232-234	244(14,300)	386(M^+); 368(M-H_2O); 353 M-(H_2O+CH_3); 267 M-(H_2O+SC)(100%)
IId	8	29	237-240	246(15,500)	384(M-H_2O); 366(M-2xH_2O); 265 M-(2xH_2O+SC)(100%)
IIe	10	34	133-134	245(15,800)	384(M^+); 369(M-SC); 122(100%)
IIf	4	22	193-194	246(14,900)	400(M^+); 382(M-H_2O); 367 M-(H_2O+CH_3) 267 M-(H_2O+SC); 122(100%)
IIg	10	31	140-141	246(15,900)	400(M^+); 382(M-H_2O); 267 M-(H_2O+CH_3) 122(100%)
IIh	10	25	210-212	246(16,200)	416(M^+); 398(M-H_2O); 380(M-2xH_2O); 283 M-(H_2O+SC); 265 M-(2xH_2O+SC); 122(100%)
IIi	6	24	131-132	245(15,000)	382(M-HCO_2H); 367 M-(HCO_2H+CH_3); 267 M-(HCO_2H+SC); 122(100%)
IIj	4	33	141-143	246(13,000)	382(M-HCO_2H); 367 M-(HCO_2H+CH_3); 267 M-(HCO_2H+SC); 122(100%)
IIk	4	37	180-182	245(15,500)	454(M-HCO_2H); 408(M-2xHCO_2H); 339 M-(HCO_2H+SC); 293 M-(2xHCO_2H+SC); 122(100%)

M^+ = molecular ion; SC = side chain.

Oxidation of 4-en-3-ols (Ve-h) to 4-en-3-ones (IVe-h)

The product from the above reaction (usually 30-35 mg) was dissolved in 5 mL chloroform and stirred for 60 h with active MnO_2 prepared according to Sondheimer (8,9). The solids were then filtered off, washed 2 x 5 mL chloroform, and the combined organic phases were evaporated. TLC showed one spot, sometimes along with a very small amount of impurity.

Reduction of 4-en-3-ones (IV) by $NaBH_4$

A solution of 50 mg of each of 4-en-3-ones (IVe-h) in 2 mL pyridine was stirred with 50 mg of sodium borohydride in 5 mL of dry pyridine at room temperature for 20 h. Water was added and the milky solution was extracted with 3 x 25 mL of ethyl acetate, washed repeatedly with dil. HCl followed by $NaHCO_3$ solution and saturated NaCl; the organic layer was dried over $MgSO_4$ and the solvent was evaporated. The presence of some free acids due to hydrolysis of esters was shown by TLC. The reaction mixture was taken up in 5 mL methanol; 2.5 mL 2,2-dimethoxypropane and 0.5 mL HCl were added and the mixture was stirred over-night. After adding 1.0 g of solid Na_2CO_3, the solution was evaporated to dryness. The residue was taken up in ethyl acetate, filtered, and the solvent was evaporated. The mixtures so obtained were analyzed by HPLC (Table 3).

Lithium/Ammonia reduction of the 1,4-dien-3-ones (II)

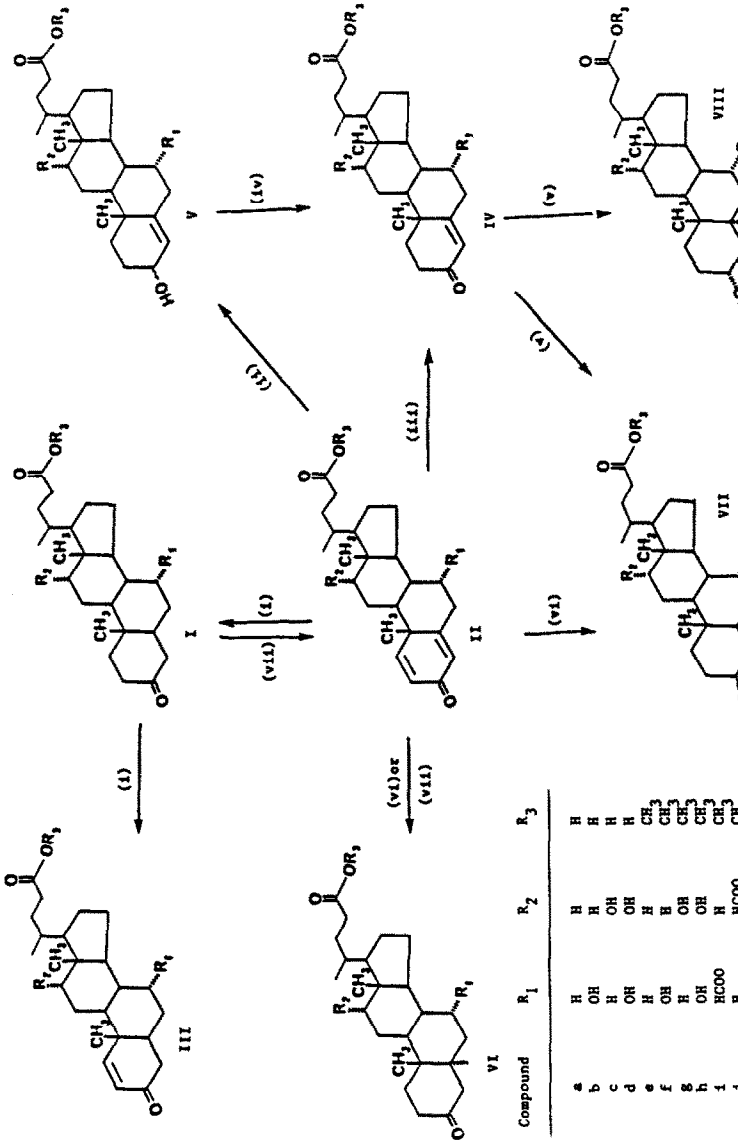
To 100 mg each of 1,4-dien-3-ones (IIa-IId) in 50 mL of anhydrous ammonia, a solution made up of 100-150 mg of lithium metal in 30 mL of ammonia was added slowly with stirring. Decolorization was achieved before subsequent addition of the reagent. Once the decolorization had slowed down appreciably (10 min or more) the rest of the lithium/ammonia solution was added and stirred for 1 h. Methanol (1 mL) was then added and the ammonia was evaporated. The residue was taken up in water, acidified with 5 N HCl, and extracted repeatedly with ethyl acetate. The combined extracts were washed once with water, dried over $MgSO_4$, and the solvent was evaporated. The mixture was reesterified as described above. A portion of the resulting mixture was separated by PLC and the zones representing different compounds were removed and extracted with acetone. The compounds were then subjected to TLC and MS analyses. Identity of each compound was established by comparison of mass spectra and R_f values with published data. The results are summarized in Table 2.

TABLE 2 PRODUCT DISTRIBUTION IN LITHIUM/AMMONIA REDUCTION OF 1,4-DIEN-3-ONES (IIa-d)

Compound	3-oxo-5α derivative VI	3β(OH)-5α derivative VII
IIb	VIb 44%	VIIb 56%
IIc	VIc 38%	VIIc 62%
IId	VId 22%	VIId 78%

RESULTS AND DISCUSSION

Methyl 3-oxo-chola-1,4-dien-24-oates (II) have generally been synthesized from the corresponding 4-en-3-ones using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as the hydrogen acceptor (7). We have observed that these compounds can also be formed from the saturated 3-oxo compounds (I) directly by refluxing with two equivalents of DDQ in dry dioxane for 4-10 h. After removal of the solvent, the resulting mixture was chromatographed on silica gel (100-200 mesh) using acetone/hexane (1:1,1:2, or 1:3 depending on the polarity of bile acid used) to give the desired 1,4-dien-3-ones in 22-37% yield. The 1-ene-3-one (III) of the bile acid derivatives was always formed in about 10-25% yield along with the desired material, indicating that the dehydrogenation took place in two steps starting with the less hindered C_1-C_2 bond. Prolonged heating or increasing the amount of DDQ did not convert the 3-oxo-compounds completely into the derivatives (II). Compounds (IIa-IIh) and their formylated derivatives (IIi-IIk) were prepared according to this procedure (Fig. 1, i).



Compound	R_1	R_2	R_3
a	H	H	H
b	OH	H	H
c	H	OH	H
d	OH	OH	H
e	H	H	CH_3
f	OH	H	CH_3
g	H	OH	CH_3
h	OH	OH	CH_3
i	HCOO	H	CH_3
j	H	HCOO	CH_3
k	HCOO	HCOO	CH_3

Figure 1. Schematic representation of preparation and reactions of various 1,4-dien-3-ones (II): (i) DDQ/dioxane, (ii) $NaBH_4$/methanol, (iii) H_2/TPPRH/benzene, (iv) MnO_2/CH_2Cl_2, (v) $NaBH_4$/pyridine, (vi) Li/NH_3, (vii) H_2/Pd or PtO_2.

Methyl esters of the bile acids gave generally better yields of 3-oxo-chola-1,4-dien-24-oates (II) as compared to the free acids. All the 1,4-dien-3-ones showed a characteristic absorption maxima at 244-246 nm (ε=13,000-16,200). Table 1 lists the different compounds prepared, the time of reaction, the yield, and major fragment ions in mass spectrometry.

Catalytic hydrogenation of the individual methyl esters (IIe-IIh) in ethyl acetate with 5% palladium-charcoal or PtO_2 provided in 3-4 h a mixture of the 3-oxo-5β- and 3-oxo-5α-compounds (Ie-Ih and VIe-VIh) (Fig. 1, vii). The identity of the components of the mixtures and their quantitation was based on GC analyses on 3% OV-1 and comparison of RRT's with authentic materials (Table 3). A third product occasionally detected in 2-4% yield was not identified. Hydrogenation of the formylated derivatives (IIi-IIk) under similar conditions resulted in partial loss of the formyl groups, especially at C-12, to produce a variety of products, and hence was not investigated further.

TABLE 3 COMPOSITION OF THE REACTION MIXTURES AFTER HYDROGENATION OF THE 1,4-DIEN-3-ONES (IIe-h)

Compound	3-oxo-5α isomer VI %	3-keto-5β isomer I %
IIe	45.0	55.0
IIf	12.0	86.0
IIg	26.0	73.0
IIh	8.0	88.0

The major product from hydrogenation over Pd/C was the 3-oxo-5β isomer except for the unsubstituted derivative IIe where nearly a 1:1 mixture of 5α- and 5β-isomers was obtained. These observations are quite in accord with the results on reduction of C-5 double bonds in steroids whereby the more stable 5β-isomer is formed preferentially (10).

The 1,4-dien-3-ones (IIe-h) were readily converted in 65 - 78% yields to the corresponding 4-en-3-ones (IVe-h) by hydrogenation in presence of tris (triphenylphosphine)-rhodium chloride (TPPRH) in dry benzene (11) (Fig. 1, iii). This conversion to the 4-en-3-ones was also accomplished in two steps by the reduction of the 1,4-diene-3-one (IIe-IIh) in 60-65% yield with sodium borohydride in methanol (Fig. 1, ii) for 4-6 h (8). Chromatography over silica gel (100-200 mesh) gave the corresponding vinyl alcohols (Ve-h) in 25-70% yields. Each vinyl alcohol was oxidized without further purification to the corresponding 4-en-3-ones (IVe-h) by stirring for 60 h with active manganese dioxide in methylene chloride (9) (Fig. 1, iv). The products were isolated in 65-80% yields.

Reduction of the 4-en-3-ones (IVe-h) to the allo acids was achieved by stirring with an excess of sodium borohydride in pyridine (12) for 20 h (Fig. 1, v). Since substantial amounts of the free acids were formed as determined by TLC on silica using acetone/hexane (1:1), the mixture of products was re-esterified

using 2,2-dimethoxypropane/methanol/HCl (3). The reaction products were then analyzed by reversed phase HPLC using a mixture of acetonitrile/acetone (Table 4) and the relative capacity factors (rk') were compared to those of authentic materials. In the case of compound (IVg) where the authentic 3β,7α-dihydroxy-5α-compound (VIIg) was not at hand, the esters (VIIg and VIIIg) were hydrolyzed to the free acid and their relative capacity factors (rk') were compared to those calculated (4). The compositions of the mixtures and the solvent systems used in HPLC are given in Table 4. As is evident from the data, the borohydride reduction is more selective than the catalytic hydrogenation. The 4-en-3-ones (IVe) and (IVg) provided nearly a 1:1 mixture of the 5α- and 5β-isomers, whereas the enones (IVf) and (IVh) produce smaller amounts of the 3α-isomer. This may be explained on the basis of hinderance of the hydride attack on C-5 by the α-OH group at C-7. In case of the 1,4-dien-3-ones (IVe) and (IVg) where the α-OH is absent, both the α- and β-side attack on C-5 are equally favorable, but in the case of compounds (IVf) and (IVh) the α-side is now more hindered and the hydride approaches the C-5 more readily from the more favorable β-side (Fig. 2).

The alcohols (VIIe-h, VIIIe-h) were separable by HPLC with the solvent mixture given in Table 4 and were converted to the corresponding 3-oxo-derivatives by the silver carbonate-Celite oxidation for identification (5).

TABLE 4 PRODUCT DISTRIBUTION IN THE $NaBH_4$ REDUCTION OF 4-ENE-3-ONES (IVe-h)

Compound	Solvent for HPLC	3α(OH)5β isomer VIII	3β(OH)5α isomer VII	Unidentified
IVe	acetonitrile: water (95:5)	49.5%	50.0%	0.5%
IVf	acetonitrile: water (75:25)	19.0%	81.0%	-
IVg	2-propanol: phosphate buffer (pH 7.0) (8:17)	47.0%	53.0%	-
IVh	acetonitrile: water (60:40)	23.0%	77.0%	-

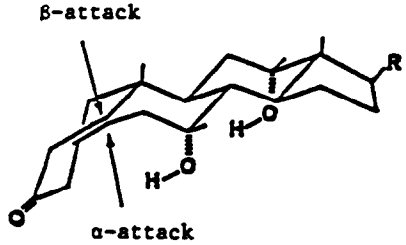


Figure 2. Stereochemical possibilities in the hydride reduction of 4-en-3-ones (IV).

Reduction of the 1,4-dien-3-ones (II) with lithium in liquid ammonia provided much better results (13). Except for the unhydroxylated derivative (IIa), which did not undergo reduction probably because of the failure to dissolve in liquid ammonia, all others were cleanly reduced to give a mixture of the corresponding 3-oxo-5α- and 3β-hydroxy-5α-compounds in reasonably good yields. Thus, from (IIb) were obtained, after esterification, methyl 3β, 7α-dihydroxy-5α-cholan-24-oate (VIIb, 56%) and methyl 3-oxo-7α-

hydroxy-5α-cholan-24-oate (VIb, 44%); from (IIc), methyl 3β,12α-dihydroxy-5α-cholanoate (VIIc, 62%) and methyl 3-oxo-12α-hydroxy-5α-cholan-24-oate (VId, 38%); and from (IId), methyl 3β,7α,12α-trihydroxy-5α-cholan-24-oate (VIId, 78%) and methyl 3-oxo-7α, 12α-dihydroxy-5α-cholan-24-oate (IVd, 22%) (Table 3). All of these compounds were identified by their mass spectra and RRTs in gas chromatography using authentic materials (14). Reduction of 3-oxo-5α-derivatives (VIb-d) with K-Selectride in dry THF produced mixtures whose major components were identified by their RRTs in gas chromatography and mass spectra as the 3α(OH)-5α-derivatives (VIIIb-d) (15,16). All of the above reactions are summarized in Figure 1.

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NOTES

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The following abbreviations have been used: TLC (thin layer chromatography), PLC (preparative layer chromatography), HPLC (high performance liquid chromatography), UV (ultra violet), allo bile acids (derivatives of 5α-cholanic acid), R_f (retention factor), RRT (relative retention time in gas chromatography), rk' (relative capacity factor in HPLC), GC (gas chromatography), MS (mass spectrometry).

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