The Deamination of the 3α- and 3β-Aminocholestanes and their Amide Derivatives

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The nitroso acetamides, naphthamides, and benzylcarbamates of 3α- and 3β-aminocholestanes were decomposed in various solvents and the nitrous acid deaminations of the free amines in acetic acid and aqueous acetic acid mixtures were studied. In these reactions the intramolecular process leads to products with high degrees of retention of configuration, whereas the intermolecular process leads to products which are predominantly equatorial. Diazoalkanes were shown not to be intermediates in these deaminations.

Les nitroso acétamides, les naphthamides, et les benzylcarbamates des amino-3α et 3β-cholestanes ont été décomposés dans plusieurs solvants. La désamination nitreuse des amines libres a été étudiée dans l'acide acétique et dans des mélanges acide-acétique – eau. Dans ces réactions, le processus intramoléculaire conduit à des produits où la rétention de configuration est très importante, alors que le processus intermoléculaire donne des produits surtout équatoriaux. Il est montré que les diazoalcanes ne sont pas des intermédiaires dans ces désaminations.

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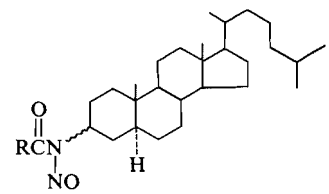
In recent years an increasing number of workers have reported on the deamination of cyclohexyl, decalyl, and steroidal amines (1–11) and have discussed the reaction paths and stereochemistry of these systems. Of these, however, only one (4) dealt with the deamination of nitrosoamides and this study was not concerned with the epimer ratios of the derived esters. It is apparent from these studies that in conformationally rigid systems few rearrangements take place with equatorial amines but are fairly common with the axial epimers. We wish to report the details of our work on the deaminations via the nitrosoacetamides, naphthamides, and benzylcarbamates of 3α- and 3β-aminocholestanes, as well as our studies of the deaminations of the free amines in acetic acid and acetic acid water mixtures. The latter studies were undertaken to reexamine the report of Shoppee *et al.* that deaminations of both isomers yielded essentially alcohols with complete retention of configuration (12).

On the basis of subsequent work (13, 14) where it has been shown that deaminations of substituted cyclohexylamines yield alcohols with retained and inverted configuration, Shoppee's results appear to be anomalous. Our results show that with both equatorial and axial amines, both alcohols are produced. This has been reported in a previous communication (15). Subsequently, Shoppee repeated his earlier work and has reported similar results (6).

The aminocholestanes were prepared by previously published procedures (16, 17) and purified by crystallization of their acetamides. The free amines were regenerated by prolonged hydrolysis in concentrated hydrochloric acid – acetic acid. The 3β-nitrosoamides (**1***a*, *b*, *c*) and the 3α-nitrosoamides (**1***a*, *c*) were prepared by the reaction of the parent amides with N_2O_4 in methylene chloride at −20 °C while the 3α-nitrosonaphthamide (**1***b*) was prepared by a low temperature modification (18). All the axial



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a R = CH_3
b R = 2-naphthyl
c R = $C_6H_5CH_2O$

TABLE 1. Decomposition of the nitrosoamides of 3α- and 3β-aminocholestanes

Compound **1**	Configuration	Solvent* + *M* equiv of addend	% olefins†	% esters†	Composition of esters 3α	3β
a	α	Ether + CH_2Cl_2	25	65	50	50
b	α	Ether‡	69	14	52	48
c	α	Hexane	84	16	83	17
c	α	Ether + 125 CH_2N_2	89	8	76	24
a	β	Cyclohexane	23	52	38	62
a	β	Cyclohexane + 42 CH_2N_2	45	44	35	65
a	β	$CHCl_3$	31	66	15	85
b	β	CH_2Cl_2 + 165 CH_3CO_2D	25	62‖	18	82
c	β	Heptane§	42	46	29	71

*All runs at room temperature unless otherwise noted.
†Corrected for recovered amide.
‡At −20 °C, $LiNO_3$ present.
§At 99 °C.
‖In addition, there was obtained 10% acetates, % composition: 25% 3α, 75% 3β.

α-nitrosoamides were unstable and decomposed at temperatures much lower than their corresponding β-epimers. The decomposition reaction mixtures were chromatographed on alumina and separated into alkene, ester, and alcohol fractions. The esters and alcohols were then analyzed by i.r. analysis, chromatographic analysis (19), gas chromatography, or by a combination of these methods. The naphthoates were converted to the alcohols via lithium aluminum hydride and analyzed as such. The reaction scheme is presented in eq. 1.

[1] $$RNHCR' \xrightarrow{N_2O_4} RN(NO)-C(=O)-R' \longrightarrow \begin{cases} ROCR' + N_2 \\ \text{Alkenes} + R'CO_2H + N_2 \end{cases}$$

The results shown in Table 1 indicate that retention of configuration predominates in all cases. The source of the inverted products is of some interest. Since these solvents are non-solvolytic, inversion must occur via an intramolecular reaction or by the reaction of some intermediate with the acid produced in the elimination reaction. The latter pathway can be checked in two ways: (*1*) by adding a scavenger for the freed carboxylic acid (in the present case diazomethane) or (*2*) by adding a large excess of a competing acid. Both of these methods were used. The addition of a large excess of diazomethane did not eliminate the inversion product. In the case of the decomposition of *N*-nitroso-3β-(2)-naphthoylamidocholestane (**1***b*) in the presence of a large excess of deuterioacetic acid, virtually no deuterium was incorporated in either the naphthoates or the acetates produced. Of these esters, only 10% was acetate and 18% of the naphthoates still had the inverted configuration. These results indicate clearly that an intramolecular inversion reaction is taking place. The mechanism of this reaction has been discussed in detail elsewhere (20). The absence of any significant deuterium incorporation in the acetates from the excess deuterioacetic acid experiment also precludes the possibility of a diazoalkane intermediate. Such intermediates are formed in non-polar solvents when the nitroso-amido group is attached to a primary carbon atom (21).

The relative unimportance of solvolysis as a source of product under these reaction conditions is shown by the low yield of acetates in the decomposition of *N*-nitroso-3β-(2)-naphthamidocholestane in the presence of a large excess of deuterioacetic acid. The ratio of acetates to naphthoates is approximately 1:6 although the ratio of acetic acid to starting nitrosonaphthamide is 165:1. Solvolysis becomes even less important when one considers that some of the

TABLE 2. The nitrous acid deamination of the 3α- and 3β-aminocholestranes

Configuration	Solvent	% olefins	% esters	% composition esters α	% composition esters β	% alcohol	% composition alcohols α	% composition alcohols β
α	Acetic acid	38	15	40	60	9	89	11
β	Acetic acid	5	53	34	66	41	10	90
α*	Acetic acid: H_2O, 1:1	45	19	—	—	13	61	39
β*	Acetic acid: H_2O:dioxane, 2:2:1	25	29	39	61	43	35	65

*Yields corrected for recovered amine.

acetate stems from frontside exchange (22), although the composition of the acetates approaches that of an equilibrium mixture with some net retention of configuration.[1]

We have found evidence for solvent bonding in runs performed in ether. A small amount (approximately 1%) of 3-ethoxycholestane of undetermined stereochemistry was found by gas chromatographic analysis of the cholestene fractions. This is in agreement with the results found by White and Billig (24) where the elements of tetrahydrofuran have been found in a nitrosoamide decomposition in this solvent. In addition to this, two other examples of solvent incorporation have been found resulting from the deamination of bridgehead amines. White *et al.* (25) have found the incorporation of the elements of benzene or chloroform during the deamination of *N*-nitroamides of norbornylamine, and Wilhelm and Curtin (26) found an 8% yield of 9-ethoxy-9,10-dihydro-9,10-ethanoanthracene from a nitrosyl chloride deamination of the corresponding amine.

We have also examined the nitrous acid deaminations of the 3α- and 3β-cholestanylamines in acetic acid and aqueous acetic acid solutions. The results are listed in Table 2. In all runs appreciable inversion is observed. The composition of the acetates does not change significantly regardless of which isomer one uses, nor over the solvent range used in our work. This data is in agreement with the revised results of Shoppee *et al.* (4, 6, 12) and with similar examples in the steroid field (5, 8, 10).

[1]An equilibrium mixture of the epimeric 4-*t*-butylcyclohexanols has been shown to consist of approximately 66% of the equatorial isomer (23). If we assume that the steric interactions involved at the 3-position of the cholestane nucleus are approximately the same, then the same equilibrium ratio should also apply here.

The source of the alcohols in anhydrous acetic acid is of some interest. Since water is produced in the reaction they could be the result of a bimolecular reaction. However, since there is such a high degree of retention of configuration in the deamination of both the 3α- and the 3β-amines it is more than likely that most of the alcohols result from an intramolecular process involving the hydroxyl group immediately associated with diazo hydroxide. In addition, deaminations in aqueous acetic acid do not significantly increase the amount of alcohols produced. These results are in agreement with those found by White and Stuber (18) and Cohen and Jankowski (3).

Products of solvolysis appear to favor the more stable 3β configuration. In all cases the acetates formed were predominantly equatorial. Also, as the concentration of water increased, the axial amine gave more of the equatorial alcohol. This is also seen in the work of Cohen and Jankowski (3) with their studies of the deamination of the axial 2-amino-*trans*-decalin in aqueous acetic acid at various concentrations. It is noteworthy in their studies as well as our own that the ratio of equatorial to axial acetate is fairly constant over a wide range of acetic acid – water mixtures. This is not true, however, in the case of the equatorial amines (27). In this case, as the water concentration increased, the ratio of equatorial to axial acetate increased, as did also the alcohol ratio to a smaller extent. From these results it is obvious that there are at least two mechanisms leading to solvolysis products. It has been suggested (3, 15) that specifically solvated ion pairs can lead to solvation products with a high degree of retention of configuration in acetic acid or in acetic acid rich aqueous reactions. In the case of the axial 2-amino-*trans*-decalin (3) and our work

this cannot apply completely since in all cases a predominance of inverted solvolysis product occurs.

Although we have examined our reactions for rearranged products, *i.e.* cholestanes substituted at the 2 and 4 positions, we have not been able to detect any significant amounts of these products. However, these rearrangements rarely exceed 5% and would not detract meaningfully from the results of this work.

Experimental

All melting points are corrected and were obtained with total immersion Anschutz thermometers. The i.r. spectra were recorded with a Perkin–Elmer model 21 spectrophotometer with scale expander. Deuterium was analyzed by combustion and i.r. comparisons. The g.l.c. measurements were performed on a Hewlett–Packard Biomedical Model 402 Gas Chromatograph.

3-Cholestanone Oxime

To a solution of 20 g of 3-cholestanone in 500 ml of refluxing ethanol was added 9.0 g of sodium acetate and 7.0 g of hydroxylamine hydrochloride. Refluxing was continued for 2 h. The solvent was then removed *in vacuo* and the residue taken up in ether, washed with water, and concentrated to dryness *in vacuo*. The residue was recrystallized from ethyl acetate. Yield 19.5 g (94%), m.p. 201–202 °C.

3α-Cholestanylamine

3α-Cholestanylamine was prepared by reduction of 3-cholestanone oxime in acetic acid over platinum according to the method of Shoppee *et al.* (16).

3β-Cholestanylamine

3β-Cholestanylamine was prepared by reduction of the oxime with sodium in refluxing amyl alcohol according to the method of Dodgson and Haworth (17).

3β-Acetamidocholestane

3β-Cholestanylamine (5.0 g) was dissolved in 150 ml of anhydrous ether and 2 ml of acetic anhydride added to the stirred solution. A precipitate formed almost immediately. The reaction was stirred for 1 h, ether then removed *in vacuo*, and the residue taken up in chloroform. The chloroform solution was washed with aqueous sodium bicarbonate and water, dried over sodium sulfate, and concentrated to dryness *in vacuo*. The amide was recrystallized from acetone to constant m.p.; m.p. 249–250°.

3α-Acetamidocholestane

This was prepared in the same manner as 3β-acetamidocholestane. Repeated recrystallization from acetone gave pure 3α-acetamidocholestane, m.p. 217–218° (lit. (28) 217–218°).

Hydrolysis of the α-Acetamidocholestane

The 3α-acetamidocholestane (1.0 g) was suspended in a mixture of 75 ml of glacial acetic acid and 30 ml of 6 *N* hydrochloric acid and refluxed for 8 days. On cooling the amine, hydrochloride precipitated as flakes. This was collected by filtration and dried *in vacuo* over P_2O_5 and KOH pellets; yield, 768 mg. Hydrolysis of 3β-acetamidocholestane was carried out in the same manner.

Benzyl-N-3α-cholestanyl Carbamate

3α-Cholestanylamine hydrochloride (1.0 g) was suspended between 40 ml of ether and 30 ml of 30% potassium hydroxide solution. The mixture was stirred until all the solid material had dissolved. Carbobenzyloxy chloride (0.41 ml) was added and stirring was continued for 2 h. The layers were then separated, the ether layer washed with water, 2*N* hydrochloric acid, water, and then dried over sodium sulfate. The ether was removed by concentration and the residue recrystallized from methanol; yield, 782 mg, m.p. 143.2–144.0 °C.

Anal. Calcd. for $C_{35}H_{55}O_2N$: C, 80.56; H, 10.62; N, 2.69. Found: C, 80.60; H, 10.39; N, 2.88.

Benzyl-N-3β-cholestanyl Carbamate

This was prepared in the same manner as the 3α-isomer. Recrystallized from ethanol; m.p. 145.0–145.5 °C.

Anal. Calcd. for $C_{35}H_{55}O_2N$: C, 80.56; H, 10.62; N, 2.69. Found: C, 80.38; H, 10.65; N, 2.85.

Benzyl-3α-cholestanyl Carbonate

3α-Cholestanol (201 mg) was dissolved in dry methylene dichloride (20 ml) and 1.0 ml of carbobenzyloxy chloride added. While stirring, a solution of 0.5 ml of dry pyridine in 12 ml of dry methylene chloride was added dropwise over a period of 2 h. The solution then stood overnight. The solution was poured into water and the organic layer washed twice with water, dried over sodium sulfate, and concentrated to dryness *in vacuo*. The residue was dissolved in a minimum amount of benzene–hexane and chromatographed on alumina (10.0 g Woelm, neutral, activity III). The carbonate (265 mg) was eluted with 5% benzene in hexane. Recrystallization from methanol–chloroform gave pure benzyl-3α-cholestanyl carbonate, m.p. 67.2–68.0 °C.

Anal. Calcd. for $C_{35}H_{54}O_3$: C, 80.41; H, 10.41. Found: C, 80.40; H, 10.56.

Benzyl-3β-cholestanyl Carbonate

This compound was prepared in the same manner as the 3α-isomer. Recrystallized from methanol, m.p. 95.2–96.2 °C.

Anal. Calcd. for $C_{35}H_{54}O_3$: C, 80.41; H, 10.41. Found: C, 80.61; H, 10.50.

3α-(2-Naphthamido)-cholestane

To 25 ml of dry pyridine was added 1.0 g of 3α-aminocholestane hydrochloride and 1.0 g of 2-naphthoyl chloride and the reaction stirred for 24 h. The reaction was then diluted with 100 ml of water and extracted with chloroform. The chloroform extract was washed with dilute sodium carbonate, water, 2 *N* hydrochloric acid, and water, then dried over sodium sulfate. Evaporation of the solvent left a residue which was recrystallized from acetone to yield the pure naphthamide; yield, 540 mg, m.p. 219.6–219.8 °C.

Anal. Calcd. for $C_{38}H_{55}ON$: C, 84.23; H, 10.23; N, 2.58. Found: C, 84.22; H, 9.97; N, 2.62.

3β-(2-Naphthamido)-cholestane

This compound was prepared in the same manner as the 3α-isomer. Recrystallized from acetone; m.p. 238.2–238.8 °C.

Anal. Calcd. for $C_{38}H_{55}ON$: C, 84.23; H, 10.23; N, 2.58. Found: C, 84.22; H, 10.12; N, 2.61.

Benzyl-N-nitroso-N-3β-cholestanyl Carbamate

Benzyl-*N*-3β-cholestanyl carbamate (398 mg) was dissolved in dry methylene chloride (10 ml) in a flask equipped with a drying tube. Anhydrous sodium acetate (300 mg) and anhydrous sodium sulfate (300 mg) were added to the solution and the mixture cooled to −20 °C in an ice-salt bath. To this was added, all at once, 10 ml of 0.325 *M* N_2O_4 in dry methylene chloride (1 ml liquid N_2O_4/50 ml CH_2Cl_2) with stirring, and the stirring continued for ½ h at −10 °C. The excess N_2O_4 was then removed *in vacuo* at 0 °C. The reaction was poured into 50 ml of ice cold concentrated sodium carbonate solution and the reaction flask washed with 50 ml of cold ether which was then added to the reaction mixture. The mixture was vigorously shaken and the layers separated. The organic layer was washed with ice cold concentrated sodium chloride solution and dried over sodium sulfate at 0 °C. After decantation the solvent was removed *in vacuo* at 0 °C. The solid yellow residue was dissolved in a minimum amount of pentane and cooled in a Dry Ice – acetone bath for several hours. The crystalline nitroso carbamate was collected by filtration–decantation and dried in hi-vac over P_2O_5; yield 372 mg, m.p. 113–114 °C (dec.).

Anal. Calcd. for $C_{35}H_{54}O_3N_2$: C, 76.32; H, 9.88; N, 5.09. Found: C, 76.45; H, 10.08; N, 5.24.

Benzyl-N-nitroso-N-3α-cholestanyl Carbamate

This compound was prepared in the same manner as the 3β-isomer. This compound could not be separated from unreacted amide and the crude nitroso-derivative was used in subsequent reactions.

N-Nitroso-3β-acetamidocholestane

This nitroso derivative was prepared in the same manner as benzyl-*N*-nitroso-3β-cholestanyl carbamate. It was recrystallized by dissolving in pentane, removing the unreacted amide by filtration, and cooling in a Dry Ice – acetone bath; yield (from 399 mg amide) 253 mg, m.p. 117.0° (dec.).

Anal. Calcd. for $C_{29}H_{50}O_2N_2$: C, 75.93; H, 10.99; N, 6.11. Found: C, 75.75; H, 10.92; N, 6.32.

N-nitroso-3β-(2-naphthamido)-cholestane

This nitroso derivative was prepared in the same manner as the nitroso carbamates. It was not possible to obtain this nitroso compound free from amide and the crude compound was used directly in decomposition reactions.

Preparation and Decomposition of N-Nitroso-3α-(2-naphthamido)-cholestane

A three-necked flask was fitted with two stopcock adapters, a rubber syringe cap, and a magnetic stirrer. One of the stopcock adapters was attached to a source of dry nitrogen. The other was alternately attached to a vacuum pump or opened when nitrogen was flowing through the system. A portion of 3α-(2-naphthamido)-cholestane (1.0 g) was dried in the flask under hy-vac for 2 h. The flask was then filled with dry nitrogen and 25 ml of ether freshly distilled from $LiAlH_4$ was added to the flask. A solution of *n*-butyllithium (1.4 ml, 1.64 *N* in hexane) was added with stirring via a syringe, after which time the solution was clear. The reaction was cooled to −60 °C in a Dry Ice – acetone bath and 50 ml of N_2O_4 gas (dried in the liquid state over P_2O_5) was added with a syringe. The solution rapidly turned yellow.

While continuing stirring, the temperature of the solution was allowed to slowly rise. At approximately −30 °C a small evolution of bubbles could be seen. At −20 °C the evolution of gas was quite evident, and a precipitate began to form. At 0 °C the evolution of gas was finished.

When the reaction had reached room temperature water was added and the layers separated. The non-aqueous layer was washed with concentrated sodium carbonate and water, dried over sodium sulfate, and concentrated to dryness *in vacuo*.

The naphthoates were isolated by chromatography on alumina and analyzed in the same manner as in the case of the decomposition of the 3β-isomer.

Preparation and Decomposition of N-Nitroso-3α-acetamidocholestane

N-Nitroso-3α-acetamidocholestane was prepared in the same manner as benzyl-*N*-nitroso-*N*-3β-cholestanyl carbamate. On the addition of ether during the work-up at 0 °C, the yellow color characteristic of the nitrosoamides disappeared completely and the reaction was then worked-up for the decomposition products. The organic layer was dried over sodium sulfate and concentrated to dryness *in vacuo*. The residue was then analyzed.

Decomposition of Nitrosoamides

The following are given as typical of the methods for decomposition of the nitrosoamides and nitrosocarbamates. All decompositions were performed at room temperature except as otherwise noted in Table 1.

Decomposition of N-Nitroso-3β-acetamidocholestane in Ether

N-Nitroso-3β-acetamidocholestane (175 mg) was dissolved in anhydrous ether (50 ml), 1 g of anhydrous sodium sulfate added, and the mixture was heated under reflux for 18 h. After this time the solution was completely colorless. The solvent was removed *in vacuo*, the residue dissolved in benzene and chromatographed on alumina (8.5 g, Woelm, neutral, activity I). Elution with benzene (45 ml) yielded 62 mg (44.0%) of mixed Δ^2- and Δ^3-cholestanes. Further elution with 2% ether in benzene (6 × 15 ml fractions) yielded 22 mg of pure 3β-cholestanyl acetate and 12 mg of mixed 3α- and 3β-cholestanyl acetates. Further elution with 20% ether in benzene eluted 18 mg of pure 3α-cholestanyl acetate; total acetates, 32.3%. Final stripping of the column with ether and 5% methanol in ether yielded 33 mg (20.2%) of material which was mostly denitrosated amide. The fractions were analyzed first by t.l.c. The composition of the mixed acetate fraction was estimated to be about 70% 3α-cholestanyl acetate by comparison with standard i.r. curves. Total recovery of material was 96.5%.

As a check the acetates were combined and analyzed by i.r. Comparison against standards showed the mixture to be 52% 3β-cholestanyl acetate and 48% 3α-cholestanyl acetate. Values from chromatography were 50% of each isomer.

Decomposition of Benzyl-N-nitroso-N-3α-cholestanyl Carbamate in Ether Containing Excess Diazomethane

Benzyl-*N*-nitroso-*N*-3α-cholestanyl carbamate (152 mg) was dissolved in a freshly prepared solution of diazomethane (approximately 1 g) in 50 ml of ether, which had been dried over KOH pellets. This stood for 48 h in the dark. The excess diazomethane and ether were then removed *in vacuo* and the residue dissolved in a minimum amount of hexane–benzene and chromatographed on alumina (15 g, Woelm,

neutral, activity III). Elution with hexane (75 ml) yielded 61 mg of mixed Δ^2- and Δ^3-cholestenes. Further elution with 2% benzene in hexane (150 ml) gave 6 mg of mixed benzyl-3α- and 3β-cholestanyl carbonates. Elution with 50% benzene–hexane yielded 54 mg of recovered carbamate. The carbonate mixture was analyzed by comparison with i.r. standards and was found to consist of 74% of benzyl-cholestan-3α-yl carbonate and 26% benzyl-cholestan-3β-yl carbonate.

Decomposition of N-Nitroso-3α-(2-naphthamido)-cholestane in O-Deuterioacetic Acid

N-Nitroso-3α-(2-naphthamido)-cholestane was prepared in the usual manner from 0.99 g of the amide. It was not isolated but was used directly. The nitroso amide was dissolved in a mixture of 40 ml of dry methylene chloride and 10 ml of 99+% *O*-deuterioacetic acid. The reaction was protected from moisture and stood for 3 days at room temperature. The solvents were then removed *in vacuo*. The residue was chromatographed on alumina (30 g, Woelm, neutral, activity II). Hexane (200 ml) eluted 131 mg of cholestenes. Further elution with 200 ml of 50% hexane–benzene gave 544 mg of mixed esters. Finally, elution with benzene yielded 203 mg of naphthamide. This amounts to a recovery of 96.5% based on starting amide.

The esters were separated by fractional sublimation at 0.06–0.03 mm Hg and 108 °C. After 3 days, all of the acetates had sublimed. The sublimation was followed by t.l.c. Analysis of the acetates (71.3 mg) was made by comparison with standards in the i.r. The mixture of acetates contained 25% 3α-cholestanyl acetate and 75% 3β-cholestanyl acetate.

The naphthoates (465 mg) were dissolved in 100 ml of dry ether and excess $LiAlH_4$ added. The reaction was heated at reflux overnight. The excess $LiAlH_4$ was decomposed with ethyl acetate, and then 6 *N* HCl added slowly until the solution was clear. The layers were separated and the ether layer washed twice with water. After drying over anhydrous sodium sulfate, the ether was removed *in vacuo*. The residue (456 mg) was then chromatographed on alumina (150 g, Alcoa F-20). Benzene eluted 59 mg of 3α-cholestanol and benzene–ether (8:1) eluted 355 mg of a mixture of 3β-cholestanol and 2-naphthyl carbinol. The naphthyl carbinol was removed by sublimation at 60° and 0.05 mm Hg. There remained 274 mg of 3β-cholestanol. Samples of the alcohols were crystallized for deuterium analysis. The acetates were used directly.

Thin-layer Chromatography Analyses

Analyses were made using silica gel G specially prepared for t.l.c. according to Stahl. Benzene was used for the moving phase and sulfuryl chloride vapors followed by steam and heating in an oven at 110° was found most satisfactory as a developing agent. Naphthoates could readily be seen with a u.v. lamp prior to sulfuryl chloride treatment. All isomeric pairs were clearly separated when sufficiently small spots of dilute solutions were applied. Only the two cholestenes did not separate. The amides did not move from the origin under these conditions but were readily analyzed by using ethyl acetate as the moving phase.

Analysis of Acetate Mixtures

(a) By Chromatography

The acetate mixture was dissolved in a small amount of benzene and placed on an alumina column (50 times the weight of acetate, Woelm, activity I, neutral). Careful elution with 5% ether in benzene separated the two acetates, the 3β-isomer being the first to come off the column. When no more 3β-isomer was present in the eluants, the remainder was eluted with ether. The composition of the fractions could be followed by t.l.c. The fractions containing each isomer were then combined and weighed.

(b) By Infrared Spectroscopy

A series of standard solutions of mixtures of 3α- and 3β-cholestanyl acetates was prepared varying in steps of 5%. The standards were dissolved in carbon tetrachloride to a final concentration of 30.0 mg/ml. The total acetate mixture from a reaction was also made up to the same concentration. Using a pair of matched 0.5 mm i.r. cells, a base line was recorded and then the reaction mixture recorded against this base line without changing any settings on the instrument. Immediately following this, standard solutions were recorded until the reaction spectrum was bracketed. The peaks used for analysis occur at 1160 and 1131 cm^{-1}. The former is characteristic of 3α-cholestanyl acetate whereas the latter is due to 3β-cholestanyl acetate. It was assumed that the absorption was linear in these small concentration variations and the value of the intermediate peaks of the reaction obtained by extrapolation.

(c) By Gas–Liquid Chromatography

Using a 4 ft × 3/8 in. pretested steroid column containing 3.8% SE-30 on AW-DMCS chromosorb W the acetates were clearly separated at 236 °C. Using an attached integrator the areas under the curves were readily calculated.

The three methods agreed very well.

Analysis of Carbonate Mixtures

A series of standards of mixed 3α- and 3β-benzylcholestanyl carbonates was prepared with a final concentration of 40 mg/ml in carbon disulfide. The spectra of these mixtures were recorded against a base line in matched 0.5 mm cells with the scale expander attachment to a Perkin–Elmer IR-21 set at 5X. Transmission *vs.* % composition was then plotted for five selected absorption peaks, 1389, 1163, 1001, 973, and 951 cm^{-1}. With the exception of the absorption at 1389 cm^{-1} the plots were essentially linear. The carbonate mixtures from the reactions were made up to the same concentration and the spectra recorded. The concentration was then estimated and the value of the 1389 cm^{-1} peak read from the graph for this concentration. The recorded 1389 cm^{-1} peak was then normalized and this factor used for the other peaks. The absorption bands at 1163 and 973 cm^{-1} were used for 3α-isomer rich solutions and those at 1001 and 951 cm^{-1} used for 3β-isomer rich solutions.

Analysis of Naphthoate Mixtures

Naphthoate mixtures were analyzed by reduction to the alcohols with $LiAlH_4$, removal of the 2-naphthyl alcohol by sublimation, and analyzing the residual cholestanols.

Analysis of 3-Cholestanol Mixtures

The cholestanol mixtures were analyzed by the chromatographic method of Vail and Wheeler (19).

Deamination of 3β-Aminocholestane in Glacial Acetic Acid

3β-Aminocholestane (prepared from 523 mg of amine hydrochloride) was dissolved in 50 ml of glacial acetic acid and 2.05 g of sodium nitrite added portionwise over a period of 2 h with stirring. The reaction was stirred overnight. Solvent was removed *in vacuo* and the residue treated

with water and ether. The layers were separated and the aqueous layer extracted twice more with ether. The combined ether layers were dried over sodium sulfate and concentrated to dryness. The residue was analyzed in the same manner as with the nitrosoacetamidocholestanes.

Deamination of 3α-Aminocholestane in Glacial Acetic Acid

The deamination of 3α-aminocholestane and the analysis of its products were similar to the method used for the 3β-isomer.

Deamination of 3β-Aminocholestane in Aqueous Acetic Acid

3β-Aminocholestane (from 433 mg of amine hydrochloride) was dissolved in a mixture of 30 ml H_2O, 40 ml acetic acid, and 20 ml of dioxane. A solution of 1.0 g of sodium nitrite in 10 ml of water was added portionwise over a period of ½ h with stirring, and stirring continued overnight. The insoluble amine nitrate (140 mg) was collected by filtration and the residue concentrated to dryness *in vacuo*. The residue was worked up as with the products from the acetic acid deamination.

Deamination of 3α-Aminocholestane in Aqueous Acetic Acid

The 3α-aminocholestane was deaminated in the same manner as the 3β-isomer except that it was dissolved in a mixture of 40 ml of water and 50 ml of acetic acid. The sodium nitrite was added in a solution of 1.0 g in 10 ml of water.

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