# THE PREPARATION OF BILE ACID AMIDES AND OXAZOLINES

Narayan K.N. Ayengar, Anil K. Singhal,
Charles K. McSherry and Erwin H. Mosbach

Department of Surgery, Beth Israel Medical Center,
New York, New York 10003

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## ABSTRACT

Amides obtained by the condensation of some bile acid chlorides with 2-amino-2-methyl-1-propanol on cyclization yield bile acid oxazolines. Physical properties of these bile acid derivatives are described. Some of the oxazolines are non-toxic and are inhibitors of 7-dehydroxylaseactivity in fecal anaerobic bacteria and purified enzymes from these bacteria.

## INTRODUCTION

The results of the recently completed National Cooperative Gallstone Study (1) indicated that under the conditions employed chenodeoxycholic acid (CDA) was relatively ineffective in achieving gallstone dissolution in man. We therefore thought it desirable to develop new compounds which would be more effective than the known cholelitholytic agents CDA and ursodeoxycholic acid (UDA). This aim can be achieved in at least three ways: First, it should be possible to synthesize analogs of CDA and UDA which would have a more intensive effect on hepatic cholesterol metabolism and secretion than the known gallstone-dissolving bile acids. Second, bile acid analogs might be developed which are more resistant to bacterial degradation and thus remain within the enterohepatic circulation for prolonged perids. Third, it should be possible to produce compounds capable of suppressing the 7-dehydroxylation of CDA or UDA, thus preserving the latter for repeated enterohepatic cycling.

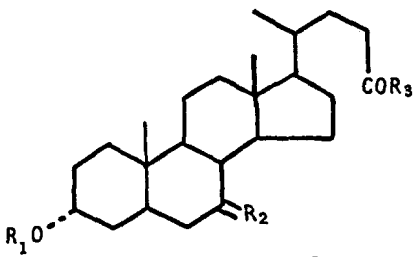
The feasibility of the third approach was already demonstrated by Mosbach and collaborators (2) who showed that in the rhesus monkey the simultaneous administration of CDA and lincomycin prevented the formation of lithocholic acid and CDA hepatotoxicity.

The oxazoline derivatives of CDA or UDA (Fig. 2) seemed suitable for preliminary investigation since they had been found to be nontoxic in animal studies (3) and had definite effects on the 7-dehydroxylation of certain bile acids in the rat. In addition, it is known that the oxazoline derivatives of dihydroxy bile acids (but not of lithocholic acid) inhibit the 7-dehydroxylation of CDA and UDA by fecal anaerobic bacteria and by purified enzymes obtained from these bacteria (4).

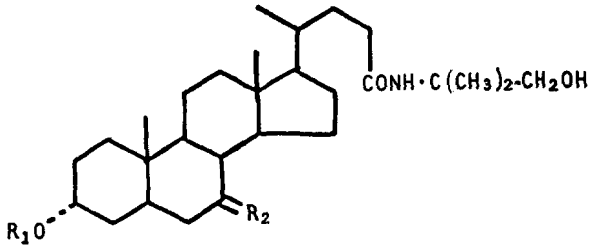
## METHODS

Melting points were determined on a Thermolyne melting point apparatus and are uncorrected. Specific rotation measurements and elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Inc., Woodside, NY. Thin layer chromatography was done on 10 x 20 cm silica gel GF plates obtained from Analtech, Newark, DE. After development of the plates the spots were visualized by spraying the heated TLC plates with 50% $H_2SO_4$ followed by phosphomolybdic acid spray. Silica gel 60 (35-70 mesh ASTM) from E. Merck, Darmstadt, Germany, was used for column chromatography. Gas chromatography was done on a Hewlett-Packard model 5830 gas chromatograph. Infrared spectra were determined on a Perkin-Elmer infrared spectrophotometer. Mass spectra were recorded on a Hewlett-Packard 5985B GC/MS at Bio/dynamics Inc., East Millstone, NJ (ion source temp: 200°C and DIP temp. profile: 50°C-350°C at 10°C per min). The structures of the compounds described in this paper are given in Figs. 1 and 2.

Preparation of 3α-formyloxy-5β-cholan-24-oic acid (IV) (6). Lithocholic acid (I) (10 g) was dissolved in 40 ml of 89.9% formic acid and 8 drops of 70% perchloric acid. The solution was heated to 55°C with stirring. The solution was cooled to 40°C and acetic anhydride (35 ml) was then added slowly maintaining the temperature

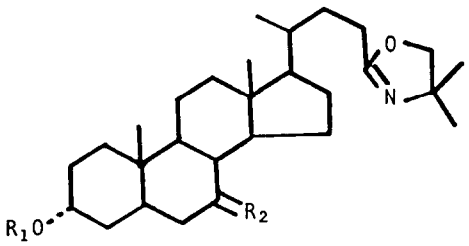



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| I | H | $H_2$ | OH |
| II | H | α-OH,β-H | OH |
| III | H | O | OH |
| IV | CHO | $H_2$ | OH |
| V | CHO | α-OCHO,β-H | OH |
| VI | CHO | O | OH |
| VII | CHO | $H_2$ | Cl |
| VIII | CHO | α-OCHO,β-H | Cl |
| IX | CHO | O | Cl |




| | $R_1$ | $R_2$ |
|---|---|---|
| X | CHO | H |
| XI | CHO | α-OCHO,β-H |
| XII | CHO | O |
| XIII | H | O |

FIG. 1




| | $R_1$ | $R_2$ | | $R_1$ | $R_2$ |
|---|---|---|---|---|---|
| XIV | CHO | $H_2$ | XVIII | H | α-OH,β-H |
| XV | CHO | α-OCHO,β-H | XIX | H | O |
| XVI | CHO | O | XX | OH | ξ-$CH_3$,ξ-OH |
| XVII | H | $H_2$ | | | |

FIG. 2

between 55-60°C. The solution was cooled and poured into 500 ml of water. The precipitate was filtered, washed with water, and dried to yield pure 3α-formyloxy-5β-cholan-24-oic acid (IV).

3α,7α-Diformyloxy-5β-cholan-24-oic acid (V) and 3α-formyloxy-7-keto-5β-cholan-24-oic acid (VI) were prepared in the same way starting from chenodeoxycholic acid (II) and 7-keto-lithocholic acid (III), respectively.

Preparation of 3α-formyloxy-5β-cholan-24-oyl chloride (VII). Freshly purified thionyl chloride (15 ml) was added dropwise with stirring to a solution of 3α-formyloxy-5β-cholan-24-oic acid (10 g) in 100 ml of benzene and the mixture was then refluxed for 4 hr. The solution was evaporated to dryness in vacuo and excess thionyl chloride was removed by distilling twice with 50 ml of benzene. The crude acid chloride VII was directly used for the next step.

The acid chlorides VIII and IX from 3α,7α-diformyloxy-5β-cholan-24-oic acid (V) and 3α-formyloxy-7-keto-5β-cholanoic acid (VI) were synthesized in the same way.

Preparation of 2-(3α-formyloxy-5β-cholan-24-amido)-2-methyl-1-propanol (X). A solution of 3α-formyloxy-5β-cholan-24-oyl chloride (VII) (10 g) in dry $CH_2Cl_2$ (40 ml) was added dropwise with stirring to a solution of 2-amino-2-methyl-1-propanol (4.25 g) in 10 ml $CH_2Cl_2$ at 0°C. At the end of 1 hr the reaction mixture was filtered and the precipitate was washed with $CH_2Cl_2$. The combined filtrate and washings were evaporated to dryness, and the crude amide was purified

by column chromatography on silica gel. The pure amide (X) was eluted with chloroform and chloroform-acetone (95:5).

2-(3α,7α-Diformyloxy-5β-cholan-24-amido)-2-methyl-1-propanol (XI) and 2-(3α-formyloxy-7-keto-5β-cholan-24-amido)-2-methyl-1-propanol (XII) were prepared in a similar manner.

Preparation of 2-(3α-hydroxy-7-keto-5β-cholan-24-amido)-2-methyl-1-propanol (XIII). To 2-(3α-formyloxy-7-keto-5β-cholan-24-amido)-2-methyl-1-propanol (XII) (10 g) in 300 ml acetone was added dropwise 0.2 N NaOH (200 ml) over a period of 30 min and the reaction mixture was then allowed to stand for 1 hr. Water (1 liter) was added, and 2-(3α-hydroxy-7-keto-5β-cholan-24-amido)-2-methyl-1-propanol separated as white crystals. The crystals were filtered, washed with water, dried and recrystallized from chloroform-acetone to obtain pure XIII.

Preparation of 2-(3α-formyloxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XIV). 2-(3α-Formyloxy-5β-cholan-24-amido)-2-methyl-1-propanol (X) (10 g) was dissolved in dry THF (50 ml), and the solution was cooled to 0°C. Ice-cold, freshly distilled thionyl chloride (8 ml) was added dropwise with stirring and the reaction mixture was allowed to stand at 0°C for 1 hr. This mixture was added slowly to 500 ml of ether, whereupon a hydrochloride precipitated which was filtered, washed with ether and dried. The free base was liberated by adding a saturated solution of $NaHCO_3$ to a suspension of hydrochloride in ether; the ether layer was washed to neutrality and dried. Evaporation of the ether gave a residue which was crystallized from acetone to get pure 2-(3α-formyloxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XIV).

2-(3α,7α-Diformyloxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XV) and 2-(3α-formyloxy-7-keto-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XVI) were also prepared as mentioned above.

Preparation of 2-(3α-hydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XVII). 2-(3α-Formyloxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XIV) (10 g) was refluxed for 2 hr with 2% methanolic sodium hydroxide (100 ml). The reaction mixture was cooled and diluted with water whereupon XVII crystallized out. The crystals were filtered, washed and dried. Recrystallization from acetone gave pure 2-(3α-hydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XVII).

Hydrolysis of 2-(3α,7α-diformyloxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XV) and 2-(3α-formyloxy-7-keto-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XVI) gave 2-(3α,7α-dihydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XVIII) and 2-(3α-hydroxy-7-keto-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XIX), respectively.

Preparation of 2-(3α,7ξ-dihydroxy-7ξ-methyl-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XX). Pure 2-(3α-hydroxy-7-keto-24-nor-5β-cholanyl-4,4-dimethyl-2-oxazoline (XIX) (15 g) was dissolved in dry benzene (240 ml) and was added dropwise with stirring to a 2.2 M solution of methyl magnesium iodide (60 ml) and benzene (60 ml) during a period of 30 min. The reaction mixture was refluxed for 6 hr and left at room temperature over night. A sat. solution of $NH_4Cl$ was added to the cold reaction mixture to decompose the complex. The reaction mixture was then extracted exhaustively with benzene. The benzene extract was washed to neutrality, dried and evaporated to dryness. The colorless oily residue was pure 2-(3α,7ξ-dihydroxy-7ξ-methyl-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline (XX) which could not be crystallized.

## RESULTS AND DISCUSSION

The syntheses of bile acid amides (X-XIII) and oxazolines (XIV-XX) were achieved by the method of Meyers and co-workers (5) with certain modifications. The nuclear hydroxy groups of the bile acid were protected by formylation according to the procedure described by Tserng and Klein (6). The formates were then treated with thionyl chloride to obtain the acid chlorides as described by Ruzicka et al. (7). The acid chlorides were condensed with 2-amino-2-methyl-1-propanol in methylene chloride, and the resulting formyloxy amides were cyclized with thionyl chloride in THF to produce the formyloxy oxazolines. Removal of the formyl group gave the free oxazolines. The oxazoline obtained from 7-ketolithocholic acid was further reacted with methyl magnesium iodide to yield the 3α,7ξ-dihydroxy-7ξ-methyl oxazoline derivative (XX).

The yields, melting points and specific rotations of the bile acid amides and oxazolines are given in Table I. Their TLC, GC and I.R. characteristics are summarized in Table II and the elemental analyses are shown in Table III.

The structures of these compounds were confirmed by infrared and mass spectral analysis. The infrared spectra (8) of the amides showed the amide band between 1620-1640 $cm^{-1}$. The bands due to the C = N linkage in the oxazolines appeared in the region 1660-1690 $cm^{-1}$. The mass spectra of the amides (X,XI,XIII) are given in Table IV. The most abundant ions were due to a fragment of the type $[CH_2=C(OH)-NH-C(CH_3)_2-CH_2OH]^+$ at m/e 131 (base peak in XIII) which is formed from the parent ion via McLafferty rearrangement. A similar cleavage is observed in the case of conjugated bile acids (9). Loss of $CH_2OH$ from the ion at m/e 131 yields the fragment $[CH_2=C(OH)-NH-C(CH_3)_2]^+$ at m/e 58 (base peak in X,XI).

The mass spectra of the oxazolines (XIV-XX) are shown in Table V. The major peaks were due to the fragments possessing the oxazoline ring. Thus the fragment at m/e 195 is due to the ion bearing the side chain along with C-15, C-16 and C-17. The ion at m/e 154 is due to the side chain which further loses 28 mass units to yield the ion at m/e 126 (base peak in XX) and is of high abundance in the other oxazolines. The base peak in the other oxazolines (XIV-XIX) appears at m/e 113 arising by a McLafferty type of rearrangement involving the oxazoline and the hydrogen at C-20 (10). In addition to these ions, fragments due to the loss of substituents from the steroidal nucleus were also observed.

TABLE I. YIELDS, MELTING POINTS AND SPECIFIC ROTATIONS OF BILE ACID DERIVATIVES

| Compound | % Yield | Melting Point | $[\alpha]_D^{25°}$ [a] |
|---|---|---|---|
| X | 98 | 153-155° | +38.6° (C, 0.33) |
| XI | 40 | 128-129° | +19.2° (C, 1.88) |
| XII | 55 | 156° | 0° (C, 0.79) |
| XIII | 64 | 212-213°; 220-222° | -23.4° (C, 0.79) |
| XIV | 58 | 108-109° | +40.8° (C, 0.85) |
| XV | 94 | 90- 92° | +18.4° (C, 0.74) |
| XVI | 98 | 117-118° | - 5.7° (C, 0.80) |
| XVII | 73 | 149-150° | +30.4° (C, 0.85) |
| XVIII | 84 | 76- 80° | + 8.0° (C, 0.71) |
| XIX | 92 | 149-150° | -26.5° (C, 0.79) |
| XX | 90 | 71- 73° | +28.1° (C, 0.78) |

[a] $\alpha_D$ determined in ethanol. C = concentration in g/100 ml.

TABLE II. $R_f$ VALUES, RELATIVE RETENTION TIMES AND I.R. SPECTRAL CHARACTERISTICS OF BILE ACID DERIVATIVES

| Compound | TLC[a] $R_f$ | Relative retention times on GC[b,c] | I.R. (Nujol) $cm^{-1}$ |
|---|---|---|---|
| X | 0.61 | 8.62[d] | 3240 (OH)<br>1730 (C=O)<br>1645 (CONH) |
| XI | 0.49 | 14.38[d] | 3340 (OH)<br>1730, 1710 (C=O)<br>1640 (-CONH) |
| XII | 0.50 | 13.14[d] | 3380, 3200 (OH)<br>1710 (C=O)<br>1640 (CONH) |
| XIII | 0.20 | 6.32[d] | 3420, 3310 (OH)<br>1620 (CONH)<br>1710 (C=O) |
| XIV | 0.81 | 3.36 | 1730 (C=O)<br>1670 (C=N) |
| XV | 0.75 | 5.66 | 1715, 1705 (C=O)<br>1660 (C=N) |
| XVI | 0.74 | 5.03 | 1730, 1710 (C=O)<br>1665 (C=N) |
| XVII | 0.58 | 3.07[d] | 1690 (C=N) |
| XVIII | 0.13 | 3.68[d] | 3340 (OH)<br>1660 (C=N) |
| XIX | 0.37 | 4.68[d] | 3300 (OH)<br>1710 (C=O)<br>1660 (C=N) |
| XX | 0.16 | 4.85[d] | 3360 (OH)<br>1660 (C=N) |

[a] Benzene:acetone (70:30).

[b] 6', 3% SE-30 column. Column temp. 260°; injector and FID temp. 280°.

[c] Retention time of 5α-cholestane = 3.08'.

[d] TMS-derivative.

TABLE III. ELEMENTAL ANALYSES OF BILE ACID DERIVATIVES

| Compound | Mol. wt. | Molecular formula | Elemental analyses | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Calculated % | | | Found % | | |
| | | | C | H | N | C | H | N |
| X | 475 | $C_{29}H_{49}O_4N$ | 73.2 | 10.31 | 2.94 | 73.85 | 10.52 | 2.79 |
| XI | 519 | $C_{30}H_{49}O_6N$ | 69.36 | 9.44 | 2.69 | 69.25 | 9.73 | 2.19 |
| XII | 489 | $C_{29}H_{47}O_5N$ | 70.99 | 9.58 | 2.85 | 71.25 | 9.84 | 2.93 |
| XIII | 461 | $C_{28}H_{47}O_4N$ | 72.87 | 10.19 | 3.03 | 72.07 | 9.94 | 3.02 |
| XIV | 457 | $C_{29}H_{47}O_3N$ | 76.14 | 10.28 | 3.06 | 76.49 | 10.46 | 3.06 |
| XV | 501 | $C_{30}H_{47}O_5N$ | 71.85 | 9.38 | 2.79 | 72.17 | 9.58 | 3.07 |
| XVI | 471 | $C_{29}H_{45}O_4N$ | 73.88 | 9.55 | 2.97 | 73.61 | 9.97 | 2.81 |
| XVII | 429 | $C_{28}H_{47}O_2N$ | 78.32 | 10.95 | 3.26 | 78.49 | 11.28 | 3.07 |
| XVIII | 445 | $C_{28}H_{47}O_3N$ | 75.51 | 10.56 | 3.14 | 75.72 | 10.76 | 3.16 |
| XIX | 443 | $C_{28}H_{45}O_3N$ | 75.85 | 10.16 | 3.16 | 75.52 | 10.37 | 3.25 |
| XX | 459 | $C_{29}H_{49}O_3N$ | 75.81 | 10.68 | 3.05 | 75.75 | 10.88 | 3.33 |

TABLE IV. RELATIVE INTENSITIES OF FRAGMENTATION IONS OF BILE ACID AMIDES

| Assignment | X | | XI | | XIII | |
|---|---|---|---|---|---|---|
| | m/e | % | m/e | % | m/e | % |
| $M^+$ | - | - | - | - | 461 | 1.7 |
| $M-CH_3$ | 460 | 0.3 | 504 | 0.8 | 446 | 6.2 |
| $M-CH_2OH$ | 444 | 1.3 | 488 | 12.2 | 430 | 14.0 |
| $M-(CH_3+R_1OH)$ | 414 | 0.4 | 458 | 1.2 | 428 | 3.8 |
| $M-(CH_3+R_1OH+R_2OH)$ | - | - | 412 | 2.9 | - | - |
| Side chain + C-15+C-16+C-17 | 213 | 0.5 | 213 | 4.1 | 213 | 3.0 |
| Side chain + C-16+C-17 | 199 | 0.9 | 199 | 3.9 | 199 | 2.8 |
| Side chain + C-17 | 185 | 0.6 | 185 | 3.2 | 185 | 2.3 |
| Side chain | 172 | 2.2 | 172 | 4.1 | 172 | 2.8 |
| Side chain - (C-20+C-21) | 144 | 23.9 | 144 | 32.7 | 144 | 69.0 |
| $[CH_2=C(OH)-NH-C(CH_3)_2-CH_2OH]^+$ | 131 | 49.3 | 131 | 99.5 | 131 | 100 |
| $[CH_2=C(OH)-NH-C(CH_3)_2]^+$ | 100 | 8.5 | 100 | 14.9 | 100 | 15.4 |
| $[CH_2=C(OH)-NH]^+$ | 58 | 100 | 58 | 100 | 58 | 89.5 |

TABLE V. RELATIVE INTENSITIES OF FRAGMENT IONS OF BILE ACID OXAZOLINES

| Assignment | XIV | | XV | | XVI | | XVII | | XVIII | | XIV | | XX | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | m/e | % | m/e | % | m/e | % | m/e | % | m/e | % | m/e | % | m/e | % |
| $M^+$ | 457 | 0.5 | 501 | 0.8 | 471 | 0.9 | 429 | 0.5 | 445 | 0.9 | 443 | 1.5 | 459 | 2.4 |
| M-$CH_3$ | 442 | 1.4 | 486 | 5.1 | 456 | 6.7 | 414 | 0.7 | 430 | 4.0 | 428 | 9.0 | 444 | 3.8 |
| M-($CH_3$+$CH_3CN$+H) | 400 | 2.6 | 444 | 7.4 | 414 | 4.2 | 372 | 2.1 | 388 | 7.5 | 386 | 7.0 | 402 | 12.8 |
| M-($CH_3$+$R_1OH$) | 396 | 1.2 | 440 | 1.7 | 410 | 2.6 | 396 | 0.6 | 412 | 1.2 | 410 | 0.3 | 426 | 3.9 |
| M-($CH_3$+$R_1OH$+$R_2OH$) | - | - | 394 | 3.7 | - | - | - | - | 394 | 0.5 | - | - | 408 | 0.3 |
| Side chain + C-15+C-16+C-17 | 195 | 0.6 | 195 | 2.7 | 195 | 7.7 | 195 | 0.8 | 195 | 0.8 | 195 | 5.7 | 195 | 1.7 |
| Side chain | 154 | 5.2 | 154 | 8.5 | 154 | 4.4 | 154 | 5.1 | 154 | 3.9 | 154 | 3.8 | 154 | 4.5 |
| Side chain - (C-20+C-21) | 126 | 89.2 | 126 | 72.6 | 126 | 87.7 | 126 | 91.2 | 126 | 82.1 | 126 | 80.5 | 126 | 100 |
| Side chain - (C-20+C-21+C-22+H) | 113 | 100 | 113 | 100 | 113 | 100 | 113 | 100 | 113 | 100 | 113 | 100 | 113 | 97.9 |
| Side chain - (C-20+C-21+C-22+C-23) | 98 | 6.7 | 98 | 7.0 | 98 | 5.8 | 98 | 6.9 | 98 | 6.6 | 98 | 7.8 | 98 | 7.0 |

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