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POTENTIAL BILE ACID METABOLITES. 1. THE EPIMERIC 3ξ,7α,12β-TRIHYDROXY-5β-CHOLANIC ACIDS. RANEY NICKEL AS AN EPIMERIZING CATALYST.

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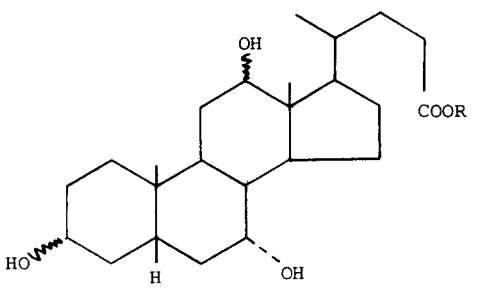
The epimeric 3α,7α,12β- and 3β,7α,12β-trihydroxy-5β-cholanic acids have been prepared. Under ambient hydrogenation conditions with Raney nickel as catalyst the axial 3β-ester (4b) is epimerized to the 3α-compound (2b).

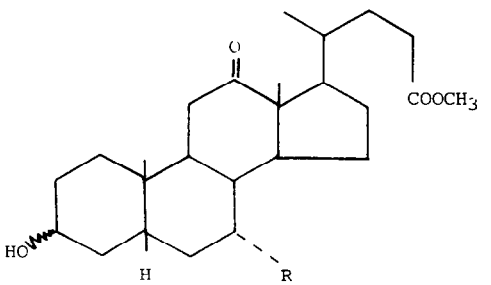
Since bile acids seem to be implicated in the development of colon cancer in humans,[1] the unreported possible metabolites of the primary bile acids are of interest. Among these, the 3α,7α,12β- and 3β,7α,12β-trihydroxy cholanic acids (2a and 4a), two of the seven possible diastereomers (5β-series[2]) of cholic acid (1a), were expected to be accessible by straight-



$(OH)_3$	R=H	$R=CH_3$
3α,7α,12α	1a	1b
3α,7α,12β	2a	2b
3β,7α,12α	3a	3b
3β,7α,12β	4a	4b

forward catalytic hydrogenation of the respective 3α-(and 3β-),7α-dihydroxy-12-oxo esters, 5 and 6, the method used in the preparation[3] of the two 12β-stereoisomers of deoxycholic (3α,-12α-dihydrocholanic) acid.

Raney nickel hydrogenation of methyl 3α,7α-dihydroxy-12-oxo cholanate[4] (5) did yield the expected products 1b and 2b.[5,6] However, hydrogenation of the epimeric 6 afforded four trihydroxy esters: the two expected 3β-isomers (3b and 4b) and surprisingly, also the 3α-esters (1b and 2b); inversion at C-3 had occurred. (Both hydrogenations at room temperature

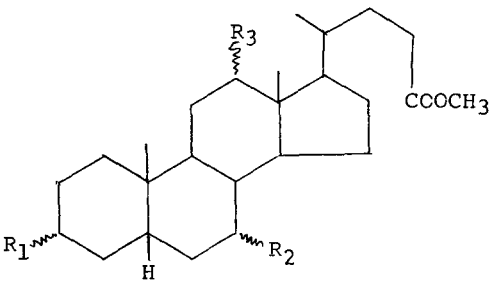


5	3α-OH, R=OH
6	3β-OH, R=OH
7	3α-OH, R=H
8	3β-OH, R=H

Methyl 3β,7α,dihydroxy-12-oxo cholanate[6,7] (6), 1.0 g, dissolved in 150 ml of methanol was shaken with 18 g of Raney nickel[8] in a Parr apparatus at hydrogen pressure of 46 lb sq. in. After 40 hr an aliquot (Fraction I) examined by NMR and HPLC showed nearly complete consumption of the ketone. Hydrogenation was resumed for an additional 22 hr; the supernatant solution was decanted, combined with the methanol washings (100 ml) of the nickel residue, and evaporated to an oil (Fraction II, 0.980 g). The nickel residue was further extracted with 50 ml of methanol by occasional stirring over a period of 24 hr; weight of extract after evaporation, 8.8 mg (Fraction III).

The CH_2Cl_2 soluble part of Fraction II (wt. 0.833 g) was chromatographed on a Florisil column and monitored by TLC. Slow elution with CH_2Cl_2-MeOH (98:2 v/v) separated the four trihydroxy cholanates[9] as follows: 3β,7α,12β-(4b), 225 mg; 3α,7α12β-(2b), 296 mg; 3β,7α,12α-(3b), 92 mg; and 3α,7α,12α-(1b), 86 mg. Compounds 1b and 3b were crystalline; 2b and 4b were not, but all four on hydrolysis yielded crystalline acids: 1a, m.p. 200°; 2a[6], m.p. 212°; 3a, m.p. 185°; 4a[6], m.p. 175°.

NMR spectroscopy offers a facile means of distinguishing and characterizing the four stereoisomers and to qualitatively follow the course of the hydrogenation. From reference spectra of the individual known monohydroxy esters, 9 to 13 inclusive, the pertinent chemical



	R_1	R_2	R_3
9	α-OH	H	H
10	β-OH	H	H
11	H	α-OH	H
12	H	H	α-OH
13	H	H	β-OH

shifts[8] and band widths of the protons at the 3, 7, and 12 positions, and the chemical shifts of the C-18 and C-19 methyl singlets were identified; these were used in characterizing the trihydroxy compounds.[10] NMR spectra of three extracts of the hydrogenation reaction (I, 40 hr aliquot; II, 62 hr main extract; III, extract after an additional 24 hr) were similar but reflected the expected changes due to the reduction of the 12-ketone and the epimerization at C-3.[11]

In previous Raney nickel hydrogenations of 12-oxo-cholanic acids[3] we have observed that differences in substituents (and their configuration at C-3) are accompanied by marked variations in the proportion of 12β to 12α alcohol formed. With compound 7 (3α-OH) the approximate ratio was ca. 1:1 whereas with the epimer 8 (3β-OH) the ratio was ca. 9:1. In this work with the analogous pair 5 and 6 the disparity between 12β:12α ratios of products is smaller but still substantial and in the same direction; for 5 (3α) the ratio is ca. 1:1, for 6 (3β) ca. 5:1.[12]

In subsequent work compound 3b under identical conditions was similarly epimerized to 1b, which shows that the 12-oxo group was not an obligatory accessory in the inversion starting with compound 6.

Racemization of steroidal alcohols at C-3 by treatment of base is well-known;[13] the reactions require alkoxides and elevated temperatures. The mechanism of racemization was shown to proceed through a transient ketone.[14] Raney nickel in boiling cymene has been reported to catalyze oxidations to ketones, epimerization (hydrogen) of steroids at C-5, and reduction of ketones when hydrogen acceptors are present.[15]

Thus, this Raney nickel-catalyzed reaction at room temperature becomes the mildest method to be reported for direct epimerization of an axial alcohol; the process uniquely does not involve an intermediary derivative before or after the inversion step.[16] Furthermore, the usual olefinic products accompanying inversion of hydroxyl derivatives are not found.[17] Very probably the epimerization at C-3 also involves an equilibration through a transitory ketone, and the equilibrium point is far on the side of the more stable equatorial (3α) alcohol.[18]

ACKNOWLEDGEMENTS

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REFERENCES AND FOOTNOTES

1. D. P. Burkitt, J. Nat'l Cancer Inst., 54, 3 (1975); J. H. Weisburger, B. S. Reddy, and E. L. Wynder, Abstracts 1977 Workshop on Large Bowel Cancer, p. 23 (1977).
2. All cholanic acid derivatives mentioned in this work are of the 5β-series; hence the 5β-designation will be omitted in the names.
3. F. C. Chang, N. F. Wood and W. G. Holton, J. Org. Chem., 30, 1718 (1965).
4. E. Berner, A. Lardon, and T. Reichstein, Helv. Chim. Acta, 30, 1542 (1947).
5. Attempts to confirm previous claims that this acid was obtained in 14% yield by reduction of 5 with sodium borohydride and subsequent recrystallization [K. Hasegawa, Hiroshima J. of Medical Sci., 8, 271 (1959)] yielded a product which consisted mainly of cholic acid. Very likely the reported acid was one of the numerous crystalline complexes of cholic acid.
6. All new compounds reported herein gave elemental and spectral analyses compatible with the assigned structures.
7. m.p. 170.0-170.5°, α_D 93.2° (chf).
8. Estimated weight. Raney nickel catalyst #28 (W. R. Grace) is essentially the same as catalyst W-2 (according to W. R. Grace, research personnel). The preparation of W-2 which contains about 0.6 g of catalyst per ml of settled catalyst is described in Org. Synthesis, Coll. Vol. III, p. 183.
9. Compounds 1b and 3b, as well as the respective free acids 1a and 3a are known compounds.
10. The values are in essential agreement with the composite values compiled by Zurcher [Helv. Chim. Acta., 46, 2054 (1963)] and Bhacca and Williams, (Applications of NMR Spectroscopy in Organic Chemistry, pp. 47, 77, etc., Holden-Day, Inc. 1964) but are more specific for this group of bile acid derivatives.
11. The most conspicious change is seen in the region of the C-19 angular methyl group: as reduction at C-12 and epimerization at C-3 proceed, the signal attributable to the C-19

methyl becomes increasingly complex while the C-18 signal remains essentially an unchanged singlet.

12. The 12β:12α ratio for the latter is derived from the sums of the two 12β- and the two 12α-products, respectively. The lower proportion of 12β alcohol formed in the hydrogenation of compounds 5 and 7 (as compared with 6 and 8, respectively) might be rationalized on the basis of a steric factor: examination of a molecular model of 5β-cholane with its folded A-ring shows that a 3α substituent would block approach of the catalyst to the C-11 ketone from the α-side of the molecule (B,C,D rings) more than would a corresponding 3β substituent. If this were crucial, one would expect that compound 5 with the axial 7α hydroxyl would more effectively block the underside approach of catalyst and the proportion of 12β-product should be even smaller. And since both rate and product ratio of hydrogenation at C-12 are influenced by the configuration of the substituent at C-3,[3] the relative rates of the two reactions (inversion of C-3 and hydrogenation at C-12) are pertinent. Obviously, more detailed studies are in order.
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14. W. von E. Doering and T. C. Aschner, J. Amer. Chem. Soc., 71, 838 (1949).
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16. A. K. Bose, B. Lai, W. A. Hoffman and M. S. Manhas, Tetrahderon Letters, 1619 (1973).
17. F. C. Chang and R. T. Blickenstaff, J. Am. Chem. Soc., 80, 2906 (1958); G. H. Douglas, P. S. Ellington, G. D. Meakins and R. Swindells, J. Chem. Soc., 1720 (1959); H. B. Henbest and W. R. Jackson, J. Chem. Soc., 954 (1962).
18. Further experiments carried out on steroidal alcohols indicate that the axial compounds [3α-cholestanol (5α) and 10 (5β)] undergo epimerization to a similar extent, while the corresponding equatorial alcohols [3β-cholestanol (5α) and 9 (5β)] under identical conditions showed only a minute degree of epimerization. These and other ancillary experiments will be reported in a full paper.

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