p-TOLUENESULFONIC ACID/METHANOL: MILD REAGENT FOR THE PREPARATION OF BILE ACID METHYL ESTERS.

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ABSTRACT

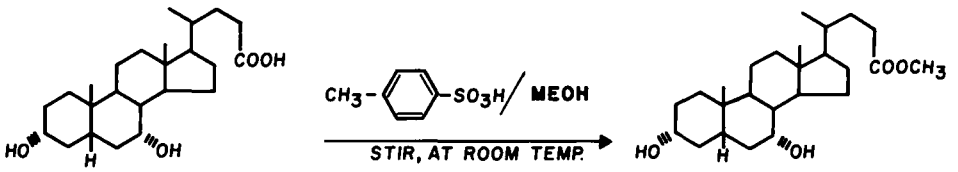
An improved method for the preparation of bile acid methyl esters is described. This is achieved by the addition of catalytic amounts of p-toluenesulfonic acid in a solution of bile acid in methanol. Advantages of this procedure over conventional methods include (1) use of a mild solid acid catalyst which prevents the formation of undesirable byproducts, (2) isolation of a solid product of high purity and (3) utilization of a relatively safe reagent in comparison to other methods involving diazomethane, hydrochloric acid or sulfuric acid.

INTRODUCTION

p-Toluenesulfonic acid has been used extensively in the literature for effecting enol etherification (1), ketalization (2), acetylation of hindered steroidal hydroxy groups (3) and enamine formation (4). Furthermore, a number of examples have been published in the literature utilizing p-toluenesulfonic acid as the catalyst in the formation of carboxylic esters (5,6).

We have found that p-toluenesulfonic acid is as effective an acid catalyst as sulfuric or hydrochloric acid for the formation of bile acid methyl esters and it is generally preferred to these mineral acids because it is less damaging to reactants and is a solid which makes it convenient to use for practical purposes. In addition, one avoids the use of diazomethane (which can be extremely toxic and hazardous) when utilizing this procedure.

Herein we describe our results with p-toluenesulfonic acid when used as a catalyst for the formation of bile acid methyl esters (Fig.1).



CHENODEOXYCHOLIC ACID

FIG 1.

CHENODEOXYCHOLIC ACID METHYL ESTER

MATERIALS AND METHODS

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, Iowa) model MP-126000, and are uncorrected.

GLC: The methyl esters, as their TMSi derivatives, were analyzed on a 180 cm x 4 mm column packed with 3% OV-17 or 1% Hi-EFF 8 BP on 80/100 mesh Gas Chrom Q; column temp. 230°C (Hewlett-Packard model 7610 gas chromatograph, Hewlett-Packard, Palo Alto, Ca.).

TLC: Performed on silica gel "G" plates (Brinkmann, 0.25 mm thickness) in the solvent system: chloroform: acetone: methanol, 70:20:2 (v/v/v). The spots were detected with phosphomolybdic acid (3.5% in isopropanol), sulfuric acid (10%) and heating for one minute at 110°C.

EXPERIMENTAL

REPRESENTATIVE PROCEDURE FOR THE PREPARATION OF BILE ACID METHYL ESTERS

This procedure was applied to all of the following bile acids: Cholic acid, chenodeoxycholic acid, ursodeoxycholic acid, hyodeoxycholic acid and lithocholic acid. Approximately the same substrate-reagent and substrate-solvent ratios were used in each case. The preparation of methyl 3α,7α-dihydroxy-5β-cholan-24-oate (methyl chenodeoxycholate) is used as an example.

To a solution of 3α,7α-dihydroxy-5β-cholan-24-oic acid (chenodeoxycholic acid) (100 mg, 0.25 mmol) in 3 ml of absolute methanol was added 10 mg (0.053 mmol) of p-toluenesulfonic acid. The solution was stirred for 2 to 2.5 hr. Completion of the reaction was monitored by TLC and GLC. Usually 80% of the reaction was complete after 1.5 to 2 hr. (Lithocholic and hyodeoxycholic acids only took 2 hr.) for complete esterification. Stirring overnight resulted in quantitative conversion. Workup of the reaction involved addition of the reaction mixture dropwise over crushed ice with stirring, thus forming a precipitate. The solid material was filtered, dried under reduced pressure and checked via mp, TLC and GLC against authentic methyl chenodeoxycholate standard. Alternatively, methanol was evaporated and residue washed once with a dilute solution of sodium bicarbonate, extracted twice with 20 ml portions of ethyl acetate, washed with water and evaporated to dryness. The residue after crystallization with acetone/ethyl acetate (90:10 v/v) gave 65 mg of the chenodeoxycholic acid methyl ester. mp(87-89°C), TLC and GLC characteristics matched those given in the literature (7,8).

DISCUSSION

Bile acid esters play an important role as synthetic organic intermediates in reaction schemes yielding other bile acid derivatives or their precursors, the bile alcohols (8-11).

Reported methods for the preparation of bile acid methyl or ethyl esters consist of dissolving the bile acid in absolute alcohol (either methanol or ethanol) and addition of a catalytic amount of concentrated mineral acid (either hydrochloric or sulfuric). Alternately, one can avoid the use of mineral acids by adopting the method utilizing diazomethane for facilitating esterification. These methods, although simple, have demonstrated drawbacks. In the former method, the use of strong mineral acids, especially hydrochloric acid, on polyhydroxy steroids can often cause the formation of undesirable artifacts. Harano et al. (12) reported the formation of dehydration products of chenodeoxycholic acid by the action of hydrochloric acid. With the use of diazomethane, dehydration products are prevented, but the formation of a methoxy ether at the 3- or 7-position can occur. Recently, Shaw et al. (13) unequivocally identified the mono-methyl ethers of methyl chenodeoxycholate formed by reaction with diazomethane as methyl 7α-hydroxy-3α-methoxy-5β-cholan-24-oate and methyl 3α-hydroxy-7α-methoxy-5β-cholan-24-oate. In addition, diazomethane is an extremely toxic, hazardous and relatively expensive substance for use in esterifying large amounts of bile acids, and its use demands extreme caution.

In contrast, esterifications with p-toluenesulfonic acid/methanol are performed under very mild conditions allowing no chance of creating hazardous conditions in the laboratory. Since p-toluensulfonic acid is a milder acid catalyst formation of unwanted artifacts can be avoided

when esterifying large amounts of bile acids, specifically chenodeoxycholic acid. Furthermore the products obtained (methyl esters) are easily isolated. We have used this reagent successfully with various kinds of bile acids (see experimental) to prepare the corresponding methyl esters. The reactions were monitored by TLC until the complete disappearance of the acid moiety was indicated.

Since much of the work in our laboratory involves the analysis of free bile acids in biological fluids (plasma, bile and feces) the use of p-toluenesulfonic acid/methanol as an esterifying reagent for the quantitative estimation of free bile acids appeared to us a safer reagent than the already existing ones (14).

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REFERENCES

1. Gannon, W.F. and House, H.O. Organic Syntheses Volumn 40, 41 (1960)
2. Rewoll, M., and Newman, M.S. Organic Syntheses Coll. Vol. 3,502 (1955).
3. Davis, M. and Petrow, V. J. Chem. Soc. 2536 (1949).
4. Hunig, S., Lucke, E., and Brenninger, W. Organic Syntheses Volumn 41, 65 (1961).
5. Allen, C.F.H. and Spangler, F.W. Organic Syntheses Coll. Vol. 3, 203 (1955).
6. Rehberg, C.E. Organic Syntheses Coll. Vol. 3, 146 (1955).
7. Nair, P.P., Kritchersky, D. (eds.): The Bile Acids (Vol. 1), Plenum Press, New York 1971.
8. Dayal, B., Bagan, E., Tint, G.S., Shefer, S., and Salen, G. Steroids 34, 259 (1979).
9. Dayal, B., Tint, G.S. and Salen, G. Steroids., 34, 581 (1979).
10. Dayal, B., Shefer, S., Tint, G.S., Salen, G. and Mosbach, E.H. J. Lipid Res. 17, 74 (1976).
11. Bose, A.K., Lal, B., Hoffman, W.A. III and Manhas, M.S. Tetrahedron Lett. 1619 (1973).
12. Harano, T., Fugita, C., Harano, K and Yamasaki, K. Steroids 30, 393 (1977).
13. Shaw, R. and Elliot, W.H. J. Lipid Res. 19, 783 (1978).
14. Salen, G., Tint, G.S., Eliav, B., Deering, N. and Mosbach, E.H. J. Clin. Invest., 53, 612 (1974).