Methanesulfonate Esters of Cholane[1] Alcohols

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Some methanesulfonate esters of cholane alcohols derived from desoxycholic acid have been prepared and submitted for screening as antitumor agents. Infrared spectra of the dimesylates have distinctive patterns in the 870 to 1000 cm.$^{-1}$ region, each of which is shown to be virtually superimposable on a composite spectrum of a pair of corresponding mono-mesylates. Antitumor screening test results indicate that the 3α,12α-, and 12α,24-compounds are inactive and harmless to test animals, but the 3α,24-isomer is quite toxic.

THE DISCOVERY that 1,4-butanediol dimesylate[2] possesses significant antitumor activity has aroused interest in the methanesulfonyloxy derivatives of other types of compounds (1, 2). Access to a number of cholanediols made available during the course of other steroidal research (3–5) led us to prepare and submit for antitumor screening their dimesylate derivatives.

Syntheses of 3α,12α-dimesyloxycholane (I), 3α,24 - dimesyloxycholane (II), 12α,24 - dimesyloxycholane (III) by conventional methanesulfonyl chloride treatment in pyridine solution of the corresponding dihydroxycholanes derivable from desoxycholic acid—3α,12α-cholanediol (IV), 3α,24-cholanediol (V), and 12α,24-cholanediol (VI), respectively—are relatively straightforward. However, the 24-mesyloxy group can undergo further reaction with chloride ion formed in the reaction, as was encountered previously in the preparation of tosylates (3), and it was found necessary to avoid excessive reaction times in the mesylation of II and III. Use of thin-layer chromatography (TLC) facilitates following the course of reaction.

As predictable from knowledge of relative acylation rates of the hydroxyl groups in bile acids (6), the axial 12α-hydroxyl, additionally hindered by the C-17 side chain and possibly by the A-ring (7), is least accessible and reacts much more slowly than hydroxyl at either 3α or 24. Thus, IV or VI in the presence of 1.5 mole equivalents of mesyl chloride after 30 minutes at room temperature are converted into 3α,12α-cholanediol 3-mesylate (VII) and 12α,24-cholanediol 24-mesylate (VIII), respectively, without trace of dimesylation, as shown by TLC.

The primary C-24 hydroxyl group probably reacts faster than the secondary 3α in the mesylation reaction, as is true in the successful selective tosylation of 3α,24-cholanediol to the 24-monotosylate (3), but under the conditions of the experiments reported here mesylation at both 3α and 24 positions proceeded too rapidly to demonstrate relative rates. In fact, when V was treated with an equimolar amount of methanesulfonyl chloride even at 0° and followed by TLC, the first spot taken immediately after mixing (zero time), when developed, showed the production of two new spots, presumably corresponding to the two possible mono-mesylates. At 2-minute reaction time, an additional spot, with R_f value of 3α,24-cholanediol dimesylate had appeared. Spots taken at 20 minutes and longer show that the reaction had stopped and no further change in concentration of the three products or of the starting diol is seen, when apparently all the methanesulfonyl chloride has been consumed. Attempts to separate the mixture by either crystallization or column chromatography have failed.[3]

Infrared spectra of the three dimesylates all have a strong sharp band near 1170 cm.$^{-1}$ and bands near 1350 cm.$^{-1}$ (Fig. 2a), which are characteristic of sulfonate esters and are attributed to $-SO_2$ stretching vibrations (8). In addition, each spectrum has a distinctive absorption pattern in the 870 to 1000 cm.$^{-1}$ region, which can be used for differentiation and easy identification of the three isomers. Study reveals that these patterns are distinctive because of the marked individuality of the isolated mesyloxyl groups in the 3α, 12α, and 24 positions in the 870 to 1000 cm.$^{-1}$ region. These characteristic absorption bands differ among themselves considerably, but each is remarkably constant in shape and intensity at least for the compounds in the cholane and cholanic acid series.[4] Thus, in this infrared region the spectra of the dimesylates

Received October 25, 1963, from the Department of Pharmacognosy, College of Pharmacy, University of Tennessee, Memphis.

Accepted for publication November 19, 1963.

This investigation was supported in part by Research Grant CA-05011 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

I am indebted to Prof. Philip C. Merker for guidance and consultation on pharmacological aspects of this work. Terry Richardson and Jo Thedford have contributed valuable technical assistance.

[1] All cholane derivatives mentioned in this paper are 5β-cholanes.

[2] Marketed as Myleran by Burroughs Wellcome & Co., Tuckahoe, N. Y.

[3] Failure by the latter method is not surprising, as R_f values by TLC of the two mono-mesylates and the dimesylate differ by little. By comparison, in the same developing system the two corresponding mono-tosylates (3) had quite different R_f values.

[4] However, mesyloxy compounds containing neighboring groups with possible interactions have not been studied.

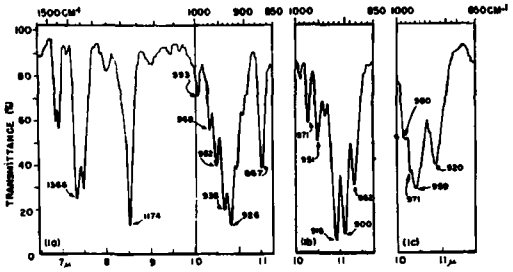


Fig. 1.—Partial infrared spectra of cholanol mesylates. (*a*) 3α-Cholanol mesylate; (*b*) 12α-cholanol mesylate; (*c*) 24-cholanol mesylate (all 4% in CCl_4).

are each virtually superimposable on a composite spectrum of a mixture consisting of the corresponding cholanol mesylates in equal proportions. Figure 1 shows partial spectra of the three cholanol mesylates: 3α-mesyloxycholane (IX), 12α-mesyloxycholane (X), and 24-mesyloxycholane (XI). Figure 2 gives spectra of the three dimesylates I, II, and III, each compared with the corresponding mixture in appropriate concentration.

The mono-mesylates VII and VIII, mentioned above, and a number of other cholane derivatives,[5] were recognized as mesylates and correctly assigned to substituent position through their distinctive infrared bands in this region.

Screening Results.—Compounds I and III in screening tests conducted by the Cancer Chemotherapy National Service Center on Sarcoma 180 in mice and lymphocytic leukemia in rats, were devoid of antitumor activity and quite harmless to test animals at daily doses of 125 mg./Kg., over a period of seven days. In contrast, compound II proved to be extremely toxic even in 0.50 mg./Kg. doses and could be administered only at low dosages. This degree of toxicity is unusual among steroids; furthermore, endocrine evaluation data, obtained from CCNSC, suggests that the 3α,24-compound may have thymolytic and antisplenic activity without marked antiflammatory effect. These results and the marked difference in biological activity between the isomeric dimesylates invites further investigations which we are pursuing.

EXPERIMENTAL

Microanalyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.; Weiler and Strauss, Oxford, England; and Alfred Bernhardt Mikroanalytisches Laboratorium, Mulheim, Germany. Melting points were taken on an electrical micro hot-stage and are uncorrected. These mesylate melting points were found to be dependent on the rate and length of heating; samples were placed on the hot-stage at one degree below the melting point, with the block temperature increasing about 0.33 degree per minute. Optical rotations were determined in 2% chloroform solution at about 25°. Infrared spectra were recorded on a Perkin-Elmer Infracord spectrophotometer (model No. 137).

Mesylation Procedure.[6]—The cholane alcohol, dried by azeotropic distillation of a benzene solution and dissolved in 7 volumes of freshly distilled pyridine, is cooled to nearly 0° in an ice bath, and while the reaction flask is twirled in the ice bath, an appropriate quantity (see below) of methanesulfonyl chloride (Eastman Kodak Co. No. 5388) is added through a pipet. After five minutes, the flask is removed from the bath and allowed to stand at room

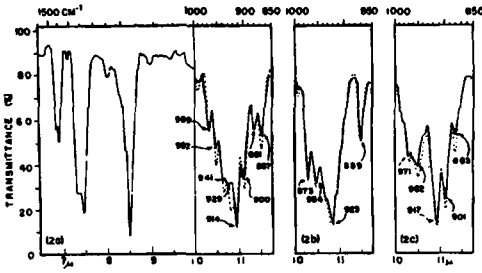


Fig. 2.—Partial infrared spectra of cholanediol dimesylates (——) and corresponding mixtures (- - -). (*a*) 3α,12α-Cholanediol dimesylate (2.5% in CCl_4); and 3α-cholanol mesylate-12α-cholanol mesylate (1:1 mixture of 4% solutions in CCl_4). (*b*) 3α,24-Cholanediol dimesylate (2.5% in $CHCl_3$); and 3α-cholanol mesylate-24-cholanol mesylate (1:1 mixture of 4% solutions in $CHCl_3$). (*c*) 12α,24-Cholanediol dimesylate (2.5% in CCl_4); and 12α-cholanol mesylate-24-cholanol mesylate (1:1 mixture of 4% solutions in CCl_4).

temperature. Crystals of pyridinium chloride separate as the reaction proceeds. The reaction can be followed readily by thin-layer chromatography (see below). When the reaction is complete, the flask is returned to the ice bath and ice chips are added, slowly to avoid undue temperature rise. In a few instances, the product crystallized at this point. Generally, processing requires transfer to a separatory funnel with ether, washing successively with cold dilute hydrochloric acid, neutralizing with dilute sodium bicarbonate solution and finally with water. After separation and treatment of the ethereal layer with a desiccant, and removal of the ether, the product is crystallized with a suitable solvent (see below).

Thin-layer Chromatography.—Plates were coated with silica gel G (Merck, Darmstad) using DeSaga equipment, developed in ligroin–ethyl acetate[7] (1:1), and sprayed with vanillin–sulfuric acid–ethanol (9).

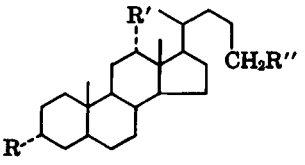
3α,12α-Cholanediol Dimesylate (I).—This was prepared from 3α,12α-cholanediol (4). Methanesulfonyl chloride used, 6 mole equivalents; reaction time, 3 hours. Crystallized from *i*-propyl ether as clear, dense prisms, m.p. 117.2 to 118.2°, $[\alpha]_D$ +

[5] Methyl 12α-mesyloxy-3-cholenate, methyl 12α-mesyloxy cholanate, methyl 3α,12α-dimesyloxycholanate, 3β-chloro-5-cholene-24-ol mesylate, 3β-chloro-11-cholene-24-ol mesylate [all described in (5)].

[6] Procedure used in the preparation of all mesylate esters described in this paper. Variations were only in proportion of methanesulfonyl chloride used, and in reaction time; these are stated under the individual compound descriptions. In general, yields obtained were nearly quantitative.

[7] Ligroin marketed as Skellysolve B by Skelly Oil Co., m.p. 63–70°, purified by sulfuric acid treatment and distillation.

TABLE I.—MESYLATES[a] OF CHOLANE ALCOHOLS



	Mesylate	R	R'	R''
I	3α,12α-Cholanediol Dimesylate	OMs[b]	OMs	H
II	3α,24-Cholanediol Dimesylate	OMs	H	OMs
III	12α,24-Cholanediol Dimesylate	H	OMs	OMs
VII	3α,12α-Cholanediol 3-Mesylate	OMs	OH	H
VIII	12α,24-Cholanediol 24-Mesylate	H	OH	OMs
IX	3α-Cholanol Mesylate	OMs	H	H
X	12α-Cholanol Mesylate	H	OMs	H
XI	24-Cholanol Mesylate	H	H	OMs

[a] Mesylate = Methanesulfonate. [b] OMs = Methanesulfonyloxy group.

70.0°, $\nu_{max.}$ (CCl_4) 1339, 1174, 969, 952, 941, 929 (sh), 914, 900, 881, 867 cm.$^{-1}$.

Anal.—Calcd. for $C_{26}H_{46}O_6S_2$ (518.76): C, 60.21; H, 8.94. Found: C, 60.56; H, 8.99.

3α-24-Cholanediol Dimesylate (II).—This compound was prepared from 3α,24-cholanediol (3, 10). Three mole equivalents of methanesulfonyl chloride were used. The reaction time was 1 hour. Needles of II crystallized from ligroin, m.p. 138.9 to 141.2°, $[\alpha]_D$ + 33.5°, $\nu_{max.}$ ($CHCl_3$) 1351, 1171, 973, 954, 923, 869 cm.$^{-1}$.

Anal.—Calcd. for $C_{26}H_{46}O_6S_2$ (518.76): C, 60.21; H, 8.94. Found: C, 60.32; H, 9.22.

12α,24-Cholanediol Dimesylate (III).—This was prepared from 12α,24-cholanediol (11).[8] Six mole equivalents of methanesulfonyl chloride were used. The reaction time was 3 hours. Crystallized from ligroin, III separated as clear, thin rectangular plates, m.p. 102.1 to 103.5°, $[\alpha]_D$ + 49.4°, $\nu_{max.}$ (CCl_4) 1350, 1179, 961, 952, 917, 901, 883 cm.$^{-1}$.

Anal.—Calcd. for $C_{26}H_{46}O_6S_2$ (518.76): C, 60.21; H, 8.94. Found: C, 60.02; H, 8.69.

III is quite stable at room temperature and can be dissolved by reflux in ligroin providing the material is stirred. Overheating, even at steam bath temperatures, causes quick decomposition of the compound.

3α,12α-Cholanediol 3-Mesylate (VII).—This was prepared from 3α,12α-cholanediol (4). The amount of methanesulfonyl chloride used was 1.5 mole equivalents and the reaction time was 1 hour. VII crystallized from ligroin as thin regular hexagons, m.p. 117.2 to 119.0°, $[\alpha]_D$ + 51.3°, $\nu_{max.}$ (CS_2) 3636, 1033 (12α-OH); 1361, 1174, 968, 954, 933, 917, 870 cm.$^{-1}$.

Anal.—Calcd. for $C_{25}H_{44}O_4S$ (440.60): C, 68.15; H, 10.07. Found: C, 67.96; H, 9.99.

12α,24-Cholanediol 24-Mesylate (VIII).—This was prepared from 12α,24-cholanediol (11). The amount of methanesulfonyl chloride used was 1.5 mole equivalents, and the reaction time was 1 hour. VIII crystallized from ligroin in bundles of needles, m.p. 97.4 to 98.6°, $[\alpha]_D$ + 35.8°, $\nu_{max.}$ (CS_2) 3663, 1036 (12α-OH); 1366, 1179, 982, 971, 960, 923 cm.$^{-1}$.

Anal.—Calcd. for $C_{25}H_{44}O_4S$ (440.60): C, 68.15; H, 10.07. Found: C, 67.87; H, 9.86.

3α-Cholanol Mesylate (IX).—This was prepared from 3α-cholanol (3). The amount of methanesulfonyl chloride used was 1.5 mole equivalents and the reaction time was 1 hour. IX crystallized from *i*-propyl ether as clear prisms, m.p. 114.5 to 115.5°, $[\alpha]_D$ + 44.1°, $\nu_{max.}$ (CCl_4) 1366, 1174, 993, 968, 956, 936, 926, 867 cm.$^{-1}$.

Anal.—Calcd. for $C_{25}H_{44}O_3S$ (424.60): C, 70.71; H, 10.45. Found: C, 70.57; H, 10.26.

12α-Cholanol Mesylate (X) (5).—This was prepared from 12α-cholanol (4). The amount of methanesulfonyl chloride used was 5 mole equivalents and the reaction time was 3 hours. X crystallized from methanol as thin plates, m.p. 99.3 to 99.8°, dec., $[\alpha]_D$ + 64.7°, $\nu_{max.}$ (CCl_4) 1344, 1174, 971, 951, 916, 900, 882 cm.$^{-1}$.

Anal.—Calcd. for $C_{25}H_{44}O_3S$ (424.60): C, 70.71; H, 10.45. Found: C, 70.92; H, 10.32.

24-Cholanol Mesylate (XI).—This was prepared from 24-cholanol (3, 12). The amount of methanesulfonyl chloride used was 1.5 mole equivalents, and the reaction time was 1 hour. XI crystallized from ligroin in micro needles, m.p. 89.5 to 91.2°, $[\alpha]_D$ + 26.6°, $\nu_{max.}$ (CCl_4) 1370, 1348, 1181, 980 (sh), 959, 920 cm.$^{-1}$.

Anal.—Calcd. for $C_{25}H_{44}O_3S$ (424.60): C, 70.71; H, 10.45. Found: C, 70.88; H, 10.65.

[8] Our preparation crystallized from benzene in thick elongated hexagons, m.p. 134.9 to 135.9°, $[\alpha]_D$ + 39.1° (chf.).

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