[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Chemistry of 3,4,7-Trisubstituted Steroids

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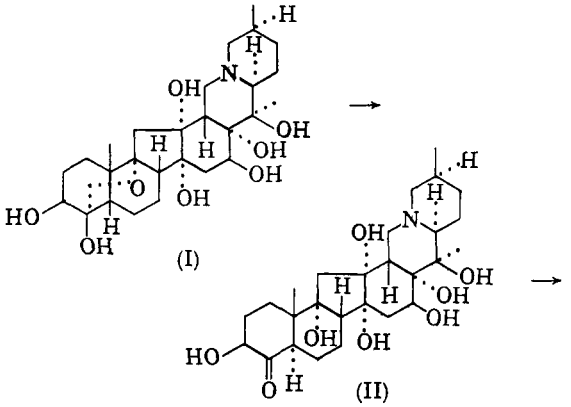
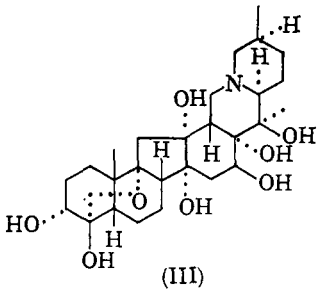
The synthesis and properties of 3,4,7-trisubstituted steroids designed as potential precursors of 3,4,7-α-ketol hemiketal derivatives are described. The contrasting behavior of cholestane-3β,7α-diol-4-one and cholestane-3β,9α-diol-4-one derivatives is discussed with regard to stereochemical stability relationships among the ceveratrum alkaloids.

The ceveratrum alkamines are characterized by a unique 3,4,9-α-ketol hemiketal system as in veracevine (I).[1-4] The alkali-catalyzed isomerizations

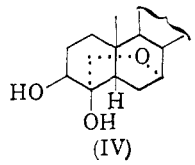


(I) (II)



(III)

which each alkamine undergo are believed to follow the pattern I–III. The alkamines germine and protoverine possess, in addition to the 9α-hydroxyl group, an α-oriented hydroxyl group at C-7.[2,4] We have indicated earlier that, whereas the bulk of the evidence strongly favors a 4,9-hemiketal structure for those derivatives having a group other than hydroxyl at C-7, *an unequivocal choice* between the 4,9-hemiketal and the 4,7-hemiketal structures for the alkamines is not possible (*cf.* footnote 27 in ref. 2). We report herein the results of an investigation undertaken to shed light on the latter problem by the preparation and study of 3,4,7-trisubstituted



(IV)

steroids designed as potential precursors of 3,4,7-α-ketol hemiketal (IV) derivatives.

The starting material for our synthesis, Δ^5-cholestene-4α-ol-3-one ethylene ketal acetate (V), was prepared by the method of Fieser and Stevenson.[5] Oxidation of V with *t*-butyl chromate (*cf.* refs. 6 and 7) afforded the desired 7-ketone, Δ^5-cholestene-4α-ol-3,7-dione 3-ethylene ketal acetate (VI) in good yield. Alkaline hydrolysis of VI afforded a product with infrared absorption indicative of absence of either an acetate ester or a hydroxyl group, but with strong absorption in the ketone region. The hydrolysis product is assigned structure VII, namely, cholestane-3,4,7-trione 3-ethylene ketal. The cholestanone (5α) configuration is preferred on the basis of the known ready isomerization of coprostane-4-one to cholestane-4-one under alkaline conditions.[8]

Hydrogenation of VI was effected in ethyl acetate at atmospheric pressure with a 10% palladium-on-charcoal catalyst. Under these conditions, hydrogenation of the double bond was slow, and only after thirty hours was the reaction complete. Careful chromatography yielded three products, cholestane-4α-ol-3,7-dione 3-ethylene ketal acetate (IX), cholestane-4α-ol-3-one ethylene ketal acetate (X), and cholestane-4α,7β-diol-3-one ethylene ketal 4-acetate (VIII). The bases for assignment of *trans* A/B configuration to the reduction products will be discussed in the sequel. Alkaline hydrolysis of IX yielded cholestane-4α-ol-3,7-dione 3-ethylene ketal (XII). Treatment of IX with ethylene glycol and boron trifluoride etherate gave cholestane-4α-ol-3,7-dione bis-(ethylene ketal) acetate (XIII). Reduction of IX with sodium borohydride in aqueous methanol gave a mixture of alcohols

(1) D. H. R. Barton, O. Jeger, V. Prelog, and R. B. Woodward, *Experientia*, **10**, 81 (1954).

(2) S. M. Kupchan and C. R. Narayanan, *J. Am. Chem. Soc.*, **81**, 1913 (1959).

(3) S. M. Kupchan, *J. Am. Chem. Soc.*, **81**, 1925 (1995).

(4) S. M. Kupchan, C. I. Ayres, M. Neeman, R. H. Hensler, T. Masamune, and S. Rajagopalan, *J. Am. Chem. Soc.*, **82**, 2242 (1960).

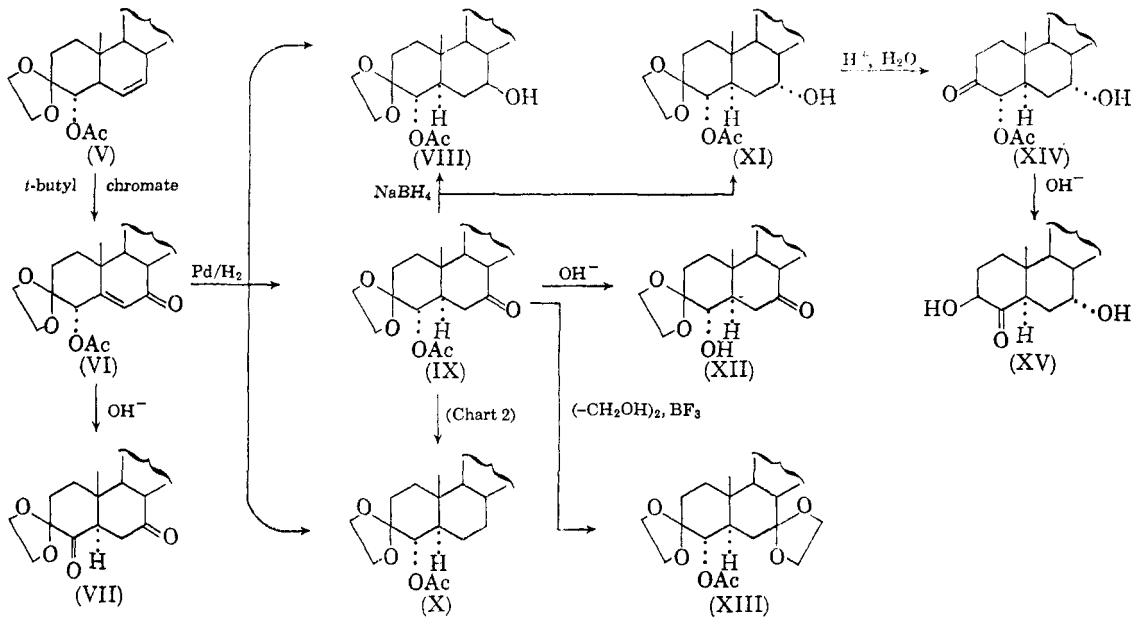
(5) L. F. Fieser and R. Stevenson, *J. Am. Chem. Soc.*, **76**, 1728 (1954).

(6) R. V. Oppenauer and H. Oberrauch, *Anales asoc. quim. arg.*, **37**, 246 (1949).

(7) L. F. Fieser, *J. Am. Chem. Soc.*, **75**, 4395 (1953).

(8) R. Stevenson and L. F. Fieser, *J. Am. Chem. Soc.*, **78**, 1409 (1956).

CHART I



epimeric at C-7. The predominating (54.5%) epimer was assigned the 7α-hydroxy structure XI, in accordance with earlier findings concerning the steric course of reduction of cholestane-7-ones with sodium borohydride in aqueous methanol.[9] The relative ease of elution of XI from the column constituted further support for assignment of the 7α-(axial)-hydroxy structure. The second, more difficultly eluted isomer (41.5%) which was found to be identical with the alcohol obtained from the catalytic hydrogenation of VI, was assigned the 7β-(equatorial)-hydroxy structure VIII. The highly stereoselective hydrogenation of the double bond of VI to *trans* A/B products (see below) indicates that the α- is much less hindered than the β-face for approach of catalyst. It is entirely reasonable, then, that the catalytic reduction of the ketone at C-7 should have proceeded to give a β-oriented hydroxyl group.

Hydrolysis of cholestane-4α,7α-diol-3-one ethylene ketal 4-acetate (XI) with hot 80% aqueous acetic acid for two and one-half hours afforded a good yield of cholestane-4α,7α-diol-3-one 4-acetate (XIV). That no acid-catalyzed isomerization to a 3-ol-4-one acetate had occurred[10] was indicated by the optical rotatory dispersion curve for XIV. The curve, with a weak positive Cotton effect, is very similar to that of cholestane-3-one,[11] but differs markedly from those of coprostane-3-one,[11] cholestane-4-one,[12] or coprostane-4-one.[13]

As discussed above, it was anticipated that the behavior on alkaline equilibration of a 3,4,7-trioxygenated steroid might parallel that of the 3,4,9-system. If this analogy proved to be correct, it was felt that alkaline treatment of XIV might lead, *via* the 3,7-diol-4-one derivative XV, to a 3,4,7-α-ketol hemiketal (IV). In fact, treatment of XIV with dilute methanolic potassium hydroxide gave an excellent yield of a carbonyl-containing product, assigned the cholestane-3β,7α-diol-4-one structure (XV). The optical rotatory dispersion curve of XV showed a very strong negative Cotton effect which compares favorably with cholestane-4-one, but resembles neither coprostane-4-one, cholestane-3-one, nor coprostane-3-one.[11] The hydroxyl group at C-3, generated under equilibrating conditions, is assigned the β-(equatorial) configuration. More vigorous alkaline treatment under a variety of conditions gave intractable mixtures, none of whose chromatographic fractions showed a carbonyl-free spectrum characteristic of a hemiketal derivative such as IV. When a solution of XV in dilute alkali was allowed to stand at room temperature, the solution developed increasing ultraviolet absorption at 320 mμ; acidification caused a shift of the maximum to 275 mμ with an accompanying increase in intensity. The latter absorption properties are characteristic of the ring A diosphenol from cevagenine.[14,15] The autoxidation of other

(9) W. G. Dauben, E. J. Blanz, Jr., J. Jiu, and R. Micheli, *J. Am. Chem. Soc.*, **78**, 3752 (1956).

(10) *Cf.* the acid-catalyzed (acid-washed alumina) isomerization of Δ^5-cholestene-4α-ol-3-one acetate to Δ^5-cholestene-3β-ol-4-one acetate, reported in ref. 5.

(11) C. Djerassi and W. Closson, *J. Am. Chem. Soc.*, **78**, 3761 (1956).

(12) C. Djerassi, W. Closson, and A. E. Lippman, *J. Am. Chem. Soc.*, **78**, 3163 (1956).

(13) C. Djerassi, R. Riniker, and B. Riniker, *J. Am. Chem. Soc.*, **78**, 6362 (1956).

(14) E. Sundt, O. Jeger, and V. Prelog, *Chem. & Ind.*, 1365 (1953).

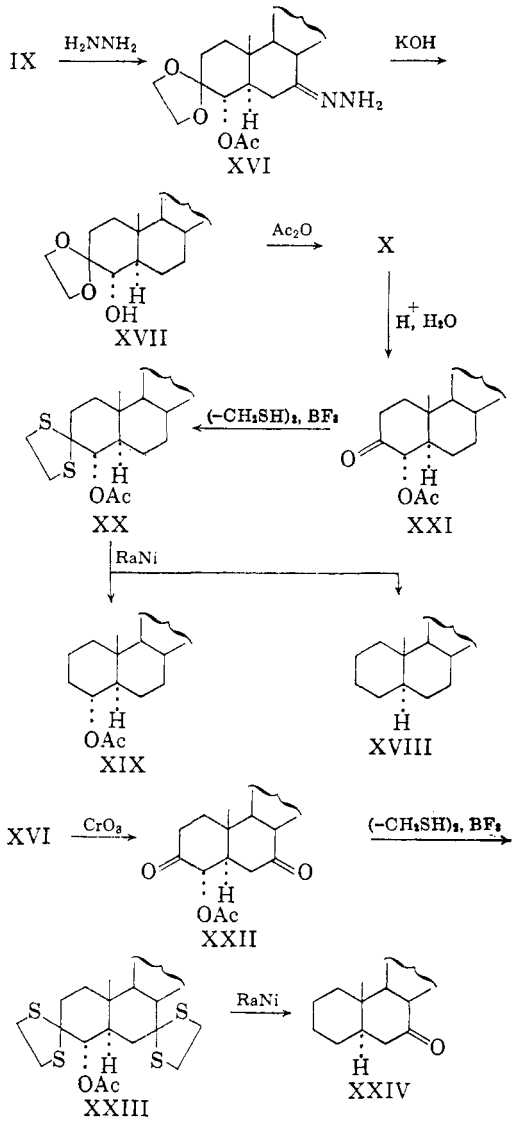
(15) *Cf.* D. H. R. Barton and J. F. Eastham, *J. Chem. Soc.*, 424 (1953).

cholestane-3β-ol-4-one derivatives to diosphenols has been observed to be equally rapid.[16]

The products from the hydrogenation of VI were degraded to establish configuration at C-5. While cholesterol, cholesteryl acetate, methyl ether and chloride, like Δ^5-cholestene, are hydrogenated almost exclusively to cholestane derivatives,[17] epicholesterol and other 3α-substituted Δ^5-cholestenes on hydrogenation give mixtures of the 5α- and 5β-dihydrides.[18] The amount of the coprostane derivative formed is roughly proportional to the size of the α-substituent. In view of the latter observations, and of the fact that VI possesses at C-3 an ethylene ketal unit with at least an —O—CH_2— segment which may be regarded as an α-axial substituent, a degradative approach to the assignment of configuration at C-5 appeared desirable. The 7-ketone (IX) was converted to its hydrazone (XVI), and the hydrazone was reduced to XVII by heating with hydrazine and sodium hydroxide in ethylene glycol under reflux. Acetylation afforded cholestane-4α-ol-3-one ethylene ketal acetate (X), identical with the product from catalytic hydrogenation. Acid hydrolysis of the ethylene ketal yielded cholestane-4α-ol-3-one acetate (XXI). Upon treatment with ethanedithiol and boron trifluoride etherate, XXI gave, in high yield, the thioketal XX, which, on treatment with Raney nickel, afforded in low yield, cholestane XVIII and cholestane-4α-ol acetate XIX. In an alternate approach, XIV was oxidized with chromic acid in acetone[19] to XXII, which was converted to its bis-ethylenethioketal derivative XXIII. Raney nickel desulfurization of XXIII afforded cholestane-7-one XXIV in small yield, as the only isolable product.

The contrasting behavior of cholestane-3β,7α-diol-4-one (XV) and cholestane-3β,9α-diol-4-one (II) derivatives upon strong alkaline treatment is of interest in connection with the question of the driving force for the cevagenine to cevine isomerization and related transformations of the type II → III. A survey of the literature leads to the conclusion that, with regard to the isomerization at the A/B ring junction, the *cis* and *trans* isomers of 9-methyldecalin derivatives are of comparable stability, and that relatively subtle and minor changes in structure can shift the equilibrium in either direction.[20-29] The molecular

CHART II



model of cevine shows that the (acidic[30]) hemiketal hydroxyl group at C-4 is situated in very convenient hydrogen bonding distance to the α-hydroxyl at C-3. The alkaline isomerization of cevagenine to cevine may be attributable to the effective hydrogen bonding stabilization of the 3α-hydroxyl-4,9-hemiketal form (III). The lower stability of veracevine (I) and its ready isomerization to cevagenine (II) may be attributable, in part, to the greater

(16) S. M. Kupchan and N. Katsui, unpublished observations.

(17) L. F. Fieser and M. Fieser, *Steroids*, Reinhold Publishing Corp., New York, 1959, p. 273.

(18) J. R. Lewis and C. W. Shoppee, *J. Chem. Soc.*, 1365 (1955).

(19) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(20) W. Hückel and E. Brinkmann, *Ann.*, **441**, 21 (1925).

(21) G. F. Davies and E. C. Gilbert, *J. Am. Chem. Soc.*, **63**, 1585 (1941).

(22) R. B. Turner, *J. Am. Chem. Soc.*, **74**, 2118 (1952).

(23) N. L. Allinger, *J. Org. Chem.*, **21**, 915 (1956).

(24) D. N. Jones, J. R. Lewis, C. W. Shoppee, and G. H. R. Summers, *J. Chem. Soc.*, 2876 (1955).

(25) D. Arigoni, J. Kalvoda, H. Heusser, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **38**, 1857 (1955).

(26) G. Stork and A. Burgstahler, *J. Am. Chem. Soc.*, **73**, 3544 (1951).

(27) A. Ross, P. A. S. Smith, and A. S. Dreiding, *J. Org. Chem.*, **20**, 905 (1955).

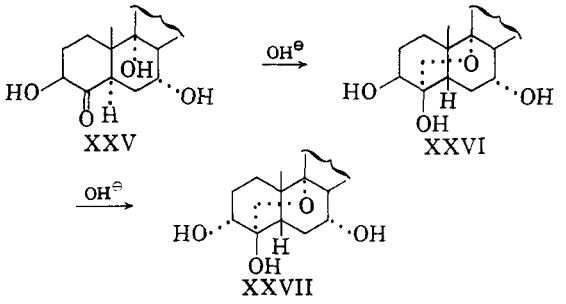
(28) W. E. Bachmann, A. Ross, A. S. Dreiding, and P. A. S. Smith, *J. Org. Chem.*, **19**, 222 (1954).

(29) F. Sondheimer and D. Rosenthal, *J. Am. Chem. Soc.*, **80**, 3995 (1958).

(30) S. Masamune, Ph.D. dissertation, University of California, 1957, *cf.* L. Michaelis, *Ber.*, **46**, 3683 (1913).

distance between the 3β-hydroxyl group from the C-4 hemiketal hydroxyl group (see model).

The view that minor changes in structure can exert profound effects on the position of the equilibrium of the ring A/B isomers is supported by earlier observations in the germine series.[2] Alkaline isomerization studies of the germine isomers showed that the order of stability is isogermine (3β,7α-dihydroxy-4-keto-9α-hydroxy-A/B *trans*, XXV) < germine (3β,7α-dihydroxy-4,9-hemiketal, XXVI) < pseudogermine (3α,7α-dihydroxy-4,9-hemiketal, XXVII). This order contrasts with those in the zygadenine and veracevine series, where the order



is 3β-hydroxy-4,9-hemiketal isomer (I) < 3β-hydroxy-4-keto-9α-hydroxy-A/B *trans* isomer (II) < 3α-hydroxy-4,9-hemiketal isomer.[1,3,31-33] Evidence was adduced in support of the view that the greater relative stability of the hemiketal forms in the germine series may be attributable to hydrogen bonding interaction of the α-oriented hydroxy group at C-7 with the α-oriented basic ethereal oxygen of the 4,9-hemiketal bridge.

Finally, the apparent failure of cholestane-3β,7α-diol-4-one (XV) to isomerize to a 3,4,7-α-ketol hemiketal IV must be considered in the light of the foregoing discussion of stereochemical stability relationships. Examination of a molecular model of the 3,4,7-α-ketol hemiketal IV reveals two factors which may play a role in making IV less stable than its analog in the 4,9-hemiketal series (III). The distortion in bond angles necessitated in order to construct a 4,7-hemiketal oxide bridge appears to increase the distance between the C-3 (α) and the C-4 hydroxyl groups relative to their juxtaposition in III. Secondly, and significantly, the model of the cage structure IV suggests that the close proximity of the α-oriented hydrogens at C-2 and C-9 would constitute a serious destabilizing interaction. Both of the foregoing factors, incidentally, would mitigate against a 3,4,7-α-ketol hemiketal structure for germine. Indeed, the extent of steric interaction between the C-2-α-hydrogen and the C-9-α-hydroxyl in the model of 3,4,7-α-ketol hemiketal structure for germine would appear to preclude that structure from serious consideration, and thereby strengthen the support for the preferred[2] 4,9-hemiketal structure XXVI.

(31) S. M. Kupchan and C. V. Deliwala, *J. Am. Chem. Soc.*, **75**, 1025 (1953).

(32) S. W. Pelletier and W. A. Jacobs, *J. Am. Chem. Soc.*, **75**, 3248 (1953).

(33) S. M. Kupchan, D. Lavie, C. V. Deliwala, and B. Y. A. Andoh, *J. Am. Chem. Soc.*, **75**, 5519 (1953).

EXPERIMENTAL[34]

Δ⁵-Cholestene-4α-ol-3,7-dione 3-ethylene ketal acetate (VI). A solution of Δ⁵-cholestene-4α-ol-3-one ethylene ketal acetate[5] (V, 40.0 g., m.p. 225–226°) in carbon tetrachloride (360 ml.) containing acetic anhydride (84 ml.) and glacial acetic acid (24 ml.) was stirred at 60° during the dropwise addition, over 1.5 hr., of a mixture of acetic anhydride (24 ml.), glacial acetic acid (84 ml.), and a freshly prepared solution of *t*-butyl chromate in carbon tetrachloride[6,7] (290 ml., equivalent to 53.0 g. of CrO_3). The mixture was stirred at 55–60° for 18 hr. and then cooled in an ice bath while a 10% oxalic acid solution (1 l.) was added slowly with vigorous stirring. Then solid oxalic acid (75.0 g.) was added, and the mixture was stirred for 1 hr. at room temperature. The organic layer was separated and the aqueous layer extracted with chloroform (3 × 650 ml.). The combined organic extracts were washed with water (2 × 2 l.), saturated sodium bicarbonate solution (1.5 l.), and water (2 l.), and dried over sodium sulfate. Filtration followed by evaporation of the solvents gave the product as a white solid which afforded Δ⁵-cholestene-4α-ol-3,7-dione 3-ethylene ketal acetate (VI) as soft fibrous needles from ether-methanol (m.p. 213–214°, 36.0 g., 88.0%). Three recrystallizations from the same solvent system gave the pure product as soft needles, m.p. 216–217°, $[\alpha]_D^{27}$ −31° (*c*, 1.68); $\lambda_{max}^{CCl_4}$ 5.78, 6.02 μ; $\lambda_{max}^{C_2H_5OH}$ 238 mμ (ε 7300).

Anal. Calcd. for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.05; H, 9.59.

Alkaline hydrolysis of Δ⁵-cholestene-4α-ol-3,7-dione 3-ethylene ketal acetate (VI). *Cholestane 3,4,7-trione 3-ethylene ketal* (VII). A solution of Δ⁵-cholestene-4α-ol-3,7-dione 3-ethylene ketal acetate (VI, 313 mg., m.p. 214–216°) in methanol (25 ml.) was treated with 10% aqueous sodium hydroxide solution (5 ml.), whereupon the solution rapidly turned yellow. The solution was heated under reflux for 75 min., poured into ice water (100 ml.), and extracted with ether (5 × 50 ml.). The ethereal extract was washed with aqueous ammonium chloride and water, dried over anhydrous sodium sulfate, and evaporated to dryness. The pale yellow solid residue (161 mg.) was recrystallized repeatedly from methanol-ether to yield cholestane-3,4,7-trione 3-ethylene ketal (VII) as white prismatic needles (115 mg., 40.0%), m.p. 235–237°, $[\alpha]_D$ −45° (*c*, 0.87); $\lambda_{max}^{CCl_4}$ 5.81, 5.90 μ.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.70; H, 9.95.

Hydrogenation of Δ⁵-cholestene-4α-ol-3,7-dione 3-ethylene ketal acetate (VI). A solution of Δ⁵-cholestene-4α-ol-3,7-dione 3-ethylene ketal acetate (VI, 10.0 g., m.p. 213–214°) in ethyl acetate (720 ml.) was added to an ethyl acetate

(34) Melting points, determined on a Fisher-Johns hot stage, are corrected. Values of $[\alpha]_D$, in chloroform unless otherwise specified, have been approximated to the nearest degree. Ultraviolet absorption spectra were determined on a Model 11 MS Cary recording spectrophotometer and 95% ethanol was used as solvent. Optical rotatory dispersions were determined in methanol solution on a Rudolph photoelectric spectropolarimeter (Model 200S). Infrared spectra were determined on solutions in chloroform on a Beckman Model IR5 spectrophotometer with NaCl prism and plates, using 0.1 mm. NaCl cells. "Petroleum ether" refers to the fraction of boiling point 60–80°. All adsorption chromatography was carried out on Merck acid-washed alumina. Microanalyses were carried out by Dr. S. M. Nagy and his associates at the Massachusetts Institute of Technology, and Mr. J. F. Alicino, Metuchen, N. J.

suspension of prereduced 10% palladium-on-charcoal (1.48 g.) and the mixture stirred under a slight positive pressure of hydrogen until uptake of gas ceased (30 hr., uptake 735 ml., *ca.* 1.5 mol. equiv.). The catalyst was removed by filtration through a short column of alumina and the solvent was evaporated, leaving a colorless solid (10.05 g., m.p. 225–235°) which showed no absorption in the ultraviolet at 238 mμ. The latter solid was chromatographed carefully on forty times its weight of alumina and three principal fractions were obtained.

Elution with benzene afforded cholestane-4α-ol-3-one ethylene ketal acetate (X) as a white solid (m.p. 230–232°, 3.41 g., 35.0%) which, after several recrystallizations from petroleum ether–benzene gave fibrous crystals, m.p. 233–234° with preliminary softening at 215°; $[\alpha]_D^{28}$ +5° (*c*, 0.44).

Anal. Calcd. for $C_{31}H_{52}O_4$: C, 76.18; H, 10.72. Found: C, 76.37; H, 10.45.

A second fraction was eluted with benzene and benzene-ether (9:1), and was characterized as cholestane-4α-ol-3,7-dione 3-ethylene ketal acetate (IX). The colorless solid (m.p. 240–243°, 5.80 g., 57.8%) was recrystallized several times from benzene–petroleum ether to yield colorless needles, m.p. 245–246°, $[\alpha]_D^{28}$ +5° (*c*, 1.30), λ_{max} 5.78, 5.85 μ. The R.D. curve,[35] of this compound showed no Cotton effect in spite of the asymmetry of the carbonyl-bearing *B* ring.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 73.98; H, 10.07.

The final fraction, eluted with ether-methanol (20:1), was a yellow solid (m.p. 238–240°, 0.815 g., 8.1%). Recrystallization from isopropyl ether yielded fibrous needles, m.p. 241–242°; $[\alpha]_D^{28}$ +35° (*c*, 0.38). This product was assigned structure VIII, namely, cholestane-4α,7β-diol-3-one 3-ethylene ketal 4-acetate.

Anal. Calcd. for $C_{31}H_{52}O_5$: C, 73.76; H, 10.38. Found: C, 73.49; H, 10.38.

Cholestane-4α-ol-3,7-dione 3-ethylene ketal (XII). A solution of cholestane-4α-ol-3,7-dione 3-ethylene ketal acetate (IX, 128 mg., m.p. 233–236°) in methanol (15 ml.) was treated with 10% aqueous sodium hydroxide solution (3 ml.). The solution was heated under reflux for 75 min., poured into ice water (50 ml.), and extracted with ether (5 × 25 ml.). The ethereal extract was washed with aqueous sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude residue (140 mg.) was recrystallized twice from petroleum ether to yield cholestane-4α-ol-3,7-dione 3-ethylene ketal (XII) as colorless needles (55 mg., 47.0%) m.p. 182–183°.

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.57; H, 10.40.

Cholestane-4α-ol-3,7-dione bis-(ethylene ketal) acetate (XIII). A solution of cholestane-4α-ol-3,7-dione 3-ethylene ketal acetate (IX, 200 mg. m.p., 238–240°) in glacial acetic acid (6 ml.) and ethylene glycol (0.6 ml.) was prepared by heating until the suspension cleared. After cooling to 38°, boron trifluoride etherate (0.4 ml.) was added, whereupon colorless needles began to separate. The mixture was allowed to stand at room temperature for 15 min. and was then cooled in ice and filtered. The product was washed with acetic acid and then methanol, and was recrystallized from methanol–acetone–ether to yield cholestane-4α-ol-3,7-dione bis-(ethylene ketal) acetate (XIII, 80 mg., 36.7%) as colorless needles, m.p. 282–285° dec., $[\alpha]_D^{26}$ +21° (*c*, 0.57).

Anal. Calcd. for $C_{33}H_{54}O_6$: C, 72.49; H, 9.96. Found: C, 72.44; H, 9.96.

Cholestane-4α,7α-diol-3-one ethylene ketal 4-acetate (XI). A solution of cholestane-4α-ol-3,7-dione 3-ethylene ketal acetate (IX, 15.90 g., m.p. 244–246°) in ether (2 l.) and methanol (1 l.) was added during 30 min. to a solution of sodium borohydride (16.25 g.) in water (150 ml.) and methanol (1.75 l.) at room temperature. The mixture was allowed to stand at room temperature with intermittent swirling for 2 hr., and was then neutralized by careful addition of 5% hydrochloric acid. Saturated sodium chloride solution (8 l.) was added, and the products were extracted with ether (6 × 900 ml.). The ethereal extract was washed, dried over anhydrous magnesium sulfate, and evaporated to dryness. The residual solid (14.88 g.) was chromatographed on alumina (500 g.). Elution with benzene gave cholestane-4α,7α-diol-3-one ethylene ketal 4-acetate (XI) (m.p. 224–226°, 8.680 g., 54.5%). Recrystallization from petroleum ether yielded long, fine needles, m.p. 226–227°, $[\alpha]_D^{28}$ +10° (*c*, 0.87).

Anal. Calcd. for $C_{31}H_{52}O_5$: C, 73.76; H, 10.38. Found: C, 73.60; H, 10.30.

Elution with ether-methanol (20:1) yielded the epimeric alcohol, cholestane-4α,7-β-diol-3-one 4-acetate (VIII) (m.p. 238–241°, 6.615 g., 41.5%). Recrystallization from isopropyl ether gave fibrous needles, m.p. 241–242°; m.p. unchanged by admixture of the product (VIII) obtained by catalytic hydrogenation. The infrared spectra of the respective samples were identical.

Cholestane-4α,7α-diol-3-one 4-acetate (XIV). A solution of cholestane-4α,7α-diol-3-one ethylene ketal 4-acetate (XI, 7.097 g., m.p. 225–227°) in 80% aqueous acetic acid (600 ml.) was heated at 100° for 2.5 hr. The bulk of the acetic acid was evaporated under reduced pressure, the concentrated solution poured into water (500 ml.), and the products were extracted with ether (3 × 300 ml.). The ethereal solution was washed with saturated sodium bicarbonate solution (2 × 1 l.) and water (2 l.), and dried over anhydrous magnesium sulfate. Evaporation of the ether left a solid residue which was crystallized from ethyl acetate to yield cholestane-4α,7α-diol-3-one 4-acetate (XIV) as colorless rods, m.p. 182–184°, 4.650 g. (71.8%), $[\alpha]_D^{28}$ −20° (*c*, 0.34), R.D. in methanol (*c*, 0.117, 25°): $[\alpha]_{650}$ −20°, $[\alpha]_{589}$ −20°, $[\alpha]_{304}$ +520°, $[\alpha]_{301}$ +610°, $[\alpha]_{265}$ −600°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.60; H, 10.50. Found: C, 75.49; H, 10.38. A second crop (0.282 g., m.p. 181–184°) was obtained from the mother liquor.

Alkaline hydrolysis of cholestane-4α,7α-diol-3-one 4-acetate (XIV). *Cholestane-3β,7α-diol-4-one* (XV). A solution of cholestane-4α,7α-diol 3-one 4-acetate (XIV, 965 mg., m.p. 182–184°) in methanol (150 ml.) was treated with a 10% methanolic solution of potassium hydroxide (10 ml.). The mixture was heated under reflux for 2 hr. and then poured into water (500 ml.). The products were extracted with ether (3 × 150 ml.), and the ethereal solution was washed with water and dried over anhydrous magnesium sulfate. Evaporation left a colorless solid (916 mg.) which was chromatographed on alumina (45 g.). Elution with benzene-ether (1:1) gave a colorless solid (m.p. 185–188°, 880 mg.). Recrystallization from petroleum ether yielded cholestane-3β,7α-diol-4-one (XV) as soft needles, m.p. 189–190°, $[\alpha]_D^{28}$ +9° (*c*, 0.30), R.D. in methanol (*c*, 0.086, 25°)[35]: $[\alpha]_{650}$ +24°, $[\alpha]_{589}$ + 24°, $[\alpha]_{304}$ −767°, $[\alpha]_{265}$ +1585°; $[\alpha]_{250}$ +1630°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.11; H, 10.91.

Treatment of XIV with 20% alcoholic potassium hydroxide on the steam bath for 30 min., with a solution of sodium in absolute alcohol under reflux for 4 hr.[36] or with sodium hydride in *t*-butyl alcohol under reflux for 4 hr. yielded intractable yellow gums with infrared peaks at 5.86, 6.00, and 6.90 μ. Careful chromatography of each of the products yielded no fractions with the carbonyl-free spectrum characteristic of a hemiketal derivative such as IV.

A solution of XV (1 mg.) in 0.2*N* alcoholic potassium hydroxide (10 ml.) was allowed to stand at room temperature for 16 hr. The ultraviolet absorption spectrum showed a maximum at 320 mμ (ε *ca.* 6000); acidification with hydrochloric acid shifted the maximum to 275 mμ with a slight increase in intensity.

(35) These optical rotatory dispersion measurements were kindly provided for us by Professor Carl Djerassi, Stanford University.

Cholestane-4α-ol-3,7-dione 3-ethylene ketal 7-hydrazone acetate (XVI). A solution of cholestane-4α-ol-3,7-dione 3-ethylene ketal acetate (IX, 540 mg., m.p. 245–247°) in boiling methanol (200 ml.) was treated with hydrazine (5.0 ml., 99%), and the mixture was heated under reflux for 5 hr. Concentration to *ca.* 50 ml. and cooling led to separation of colorless needles. Two recrystallizations from methanol yielded cholestane-4α-ol-3,7-dione 3-ethylene ketal 7-hydrazone acetate (XVI) as long needles (0.468 g., 84.3%), m.p. 253–255° dec.

Anal. Calcd. for $C_{31}H_{52}O_4N_2$: C, 72.05; H, 10.14; N, 5.42. Found: C, 72.91; H, 10.71; N, 5.86.

Cholestane-4α-ol-3-one ethylene ketal (XVII). A solution of potassium hydroxide (800 mg.) and hydrazine (5.0 ml., 99%) in ethylene glycol (40 ml.) was distilled until it refluxed freely at 185°. To the cooled solution was added cholestane-4α-ol-3,7-dione 3-ethylene ketal 7-hydrazone acetate (XVI, 460 mg., m.p. 253–255° dec.), and the mixture was heated under reflux at 185° for 8 hr. A product began to crystallize upon cooling. The mixture was poured into water (500 ml.) and the precipitate filtered. Recrystallization twice from methanol gave cholestane-4α-ol-3-one ethylene ketal (XVII) as fine needles (327 mg., 82.0%), m.p. 161–162°, $[\alpha]_D^{28}$ ±0° (*c*, 1.73).

Anal. Calcd. for $C_{29}H_{50}O_3$: C, 77.97; H, 11.28. Found: C, 77.95; H, 11.11.

Acetylation of cholestane-4α-ol-3-one ethylene ketal (XVII) *to cholestane-4α-ol-3-one ethylene ketal acetate* (X). A solution of cholestane-4α-ol-3-one ethylene ketal (XVII, 313 mg., m.p. 159–162°) in pyridine (10 ml.) and acetic anhydride (3.0 ml.) was allowed to stand overnight at room temperature. Evaporation to dryness under reduced pressure left a solid which was heated for 30 min. with methanol (50 ml.) and benzene (50 ml.). Evaporation of the solvents under reduced pressure left a solid residue which, upon crystallization from benzene, yielded cholestane-4α-ol-3-one ethylene ketal acetate (X, 300 mg., 87.5%), m.p. 226–229°. The infrared spectrum was indistinguishable from that of the material obtained by hydrogenation of Δ⁵-cholestene-4α-ol-3,7-dione ethylene ketal acetate (IX), and the melting point was not depressed by admixture of the product prepared earlier.

Acid hydrolysis of cholestane-4α-ol-3-one ethylene ketal acetate (X). A solution of cholestane-4α-3-one ethylene ketal acetate (X, 3.50 g. m.p. 231–234°) in 80% acetic acid (1.5 l.) was heated at 100° for 6 hr. The bulk of the solvent was distilled under reduced pressure and the concentrated solution was poured into water (500 ml.). The products were collected in ether (3 × 200 ml.), and the ethereal solution was washed with sodium bicarbonate solution (2 × 500 ml.) and water (500 ml.), and dried over anhydrous magnesium sulfate. Evaporation to dryness left a solid which was crystallized from methanol to yield cholestane-4α-ol-3-one acetate (XXI) in the form of rods (2.39 g., 75%), m.p. 147–148°, $[\alpha]_D^{28}$ −4° (*c*, 0.51).

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.29; H, 10.70.

The melting point was undepressed by admixture with an authentic sample of cholestane-4α-ol-3-one acetate kindly supplied by Dr. K. L. Williamson.[37] The infrared spectra of the respective samples were identical.

Cholestane-4α-ol-3-one ethylene thioketal acetate (XX). A solution of cholestane-4α-ol-3-one (XXI, 1.00 g., m.p. 147–148°) in ethane dithiol (2.0 ml.) was treated with boron trifluoride etherate (0.5 ml.). The mixture became warm and upon addition, after 5 min., of methanol (20 ml.), a white crystalline precipitate formed. Recrystallization from petroleum ether several times gave cholestane-4α-ol-3-one ethylene thioketal acetate (XX) as long needles (0.850 g., 64.0%), m.p. 217–218°, $[\alpha]_D^{28}$ +28° (*c*, 1.25).

Anal. Calcd. for $C_{31}H_{52}S_2O_2$: C, 71.50; H, 10.07. Found: C, 71.49; H, 9.88.

Desulfurization of cholestane-4α-ol-3-one ethylene thioketal acetate (XX). A solution of cholestane-4α-ol-3-one ethylene thioketal acetate (XX, 293 mg., m.p. 217–218°) in absolute ethanol (200 ml.) was treated with Raney nickel (W2, *ca.* 2 g.), and the reaction mixture was heated under reflux for 24 hr. The suspension was filtered and the filtrate was evaporated to dryness. The gummy residue (280 mg.) was chromatographed on alumina (15 g.). Elution with petroleum ether containing up to 10% benzene gave a low melting solid (85 mg.) with an infrared spectrum indicative of a saturated hydrocarbon structure. Recrystallization six times from ether-methanol gave platelets (38 mg., 17.9%), m.p. 77–78° (*cf.* cholestane,[38] m.p. 80°, and coprostane,[39] m.p. 70°). The melting point was not depressed by admixture of an authentic sample of cholestane[40] (m.p. 78–80°), and the infrared spectra of the respective samples were identical. Elution with benzene gave a crystalline fraction (107 mg.) whose infrared spectrum showed a peak at 5.79 μ. After three recrystallizations from methanol, this material was obtained as platelets (56 mg., 23.3%), m.p. 110–111° (*cf.* cholestane 4α-ol acetate,[41] m.p. 113°).

Anal. Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.65; H, 11.72.

Cholestane-4α-ol-3,7-dione acetate (XXII). A solution of cholestane-4α,7α-diol-3-one 4-acetate (XIV, 1.157 g. m.p. 182–184°) in acetone (350 ml.) was cooled in ice and treated with 8*N* chromic acid[42] (1.25 ml.). The reaction mixture was swirled for 5 min., and methanol (10 ml.) was then added to decompose excess oxidant. The bulk of the acetone was evaporated, the concentrated solution was poured into water (400 ml.), and the products were extracted with ether (3 × 100 ml.). The ethereal extract was washed with dilute sodium bicarbonate solution (500 ml.) and water (500 ml.) and dried over magnesium sulfate. Evaporation of the ether gave a colorless solid which was crystallized from methanol to yield cholestane-4α-ol-3,7-dione acetate (XXII) as white needles (840 mg., 73.0%), m.p. 185–186°, $[\alpha]_D^{28}$ −40° (*c*, 0.19).

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.13; H, 10.00.

Cholestane-4α-ol-3,7-dione bis-ethylenethioketal acetate (XXIII). A solution of cholestane-4α-ol-3,7-dione acetate (XXII, 840 mg., m.p. 185–186°) in ethanedithiol (4.0 ml.) was treated with boron trifluoride etherate (1.0 ml.). The mixture became warm and, within 2 min., assumed a semi-solid consistency. After 5 min., methanol (20 ml.) was added, and the product was filtered and recrystallized several times from petroleum ether. Cholestane-4α-ol-3,7-dione bis-ethylenethioketal (XXIII) was obtained as long needles (990 mg., 89.0%), m.p. 264–265°, $[\alpha]_D^{28}$ +23° (*c*, 1.24).

Anal. Calcd. for $C_{33}H_{54}S_4O_2$: C, 64.89; H, 8.91. Found: C, 64.86; H, 8.91.

Desulfurization of cholestane-4α-ol-3,7-dione bis-ethylene-thioketal acetate (XXIII). A solution of cholestane-4α-ol-3,7-dione bis-ethylenethioketal acetate (XXIII, 590 mg., m.p. 264–265°) in methanol (800 ml.) was treated with Raney nickel (W2, *ca.* 2.5 g.), and the mixture was heated under reflux for 24 hr. The suspension was filtered and the filtrate evaporated to dryness to yield a gummy solid (576 mg., λ_{max} 5.78, 5.86 μ), which was chromatographed on alumina (20 g.). Elution with 10% benzene in petroleum

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(38) J. Mauthner, *Monatsh.*, **30**, 635 (1909).

(39) A. Windaus and C. Uibrig, *Ber.*, **48**, 857 (1915).

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(42) The chromic acid solution was prepared by dissolving pure chromium trioxide (6.67 g.) in water, adding concentrated sulfuric acid (5.33 ml.), and diluting to 25 ml. with water.

ether gave a crystalline solid (156 mg., m.p. 70–85°). Recrystallization from methanol five times yielded cholestane-7-one XXIV as needles (32 mg., 8.0%), m.p. 115–117° $[\alpha]_D^{27}$ −51°.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.65; H, 11.96.

There was no depression in melting point upon admixture with a sample of cholestane-7-one (m.p. 118°, $[\alpha]_D$ −47°) prepared by the method of Windaus,[43] and the infrared spectra of the respective samples were identical.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ALIGARH MUSLIM UNIVERSITY]

Flower Pigments. Flavonoids from *Argemone mexicana linn.* (*Papaveraceae*)

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This paper reports details of the isolation in pure form of isorhamnetin (4′,5,7-trihydroxy-3′-methoxyflavonol) and two of its glycosides from the flowers of *Argemone mexicana*. The new glycosides have been identified as isorhamnetin-3-glucoside and isorhamnetin-7-diglucoside.

Argemone, a genus of prickly herbs, includes about twelve species. *Argemone mexicana* (English prickly poppy, Mexican poppy; Hind. Bharband, Satiyanashi) is the only species[1] found in India. It is native to tropical America but has become naturalized in India and runs wild all over the country. The yellow juice which exudes when the plant is injured has long been used in India as a medicine[2] for dropsy, jaundice, and cutaneous affections. It was also considered as a diuretic.[2] The seed oil was used in lamps and medicinally[1] in ulcers and eruptions. The seeds and the seed oil had also been employed as a remedy[2] for dysentery and other intestinal affections. The mustard oil, adulterated with argemone oil, has been established to produce symptoms resembling those of epidemic dropsy.[1]

The plant has been reported[3] to contain berberine and protopine, and the fatty acid content of the seeds has been investigated.[4] In a recent note[5] the results of our preliminary investigation of the coloring matter of the bright yellow flowers were described.

A free aglycone, m.p. 304–306°, and two glycosides, m.p. 165–167° and 208–210°, have been isolated from the flower extract of *A. mexicana*. The aglycone was found to be a flavonol by the appearance of a pink coloration on reduction[6] with magnesium and hydrochloric acid and a bright yellow coloration with Wilson boric reagent.[7] The methanolic solution of the aglycone was not oxidized by pentammine cobaltrichloride,[8] indicating the absence of two or more adjacent phenolic hydroxyl groups. Micro-Zeisel determination showed the presence of one methoxyl group. Methylation of the aglycone with dimethyl sulfate yielded a compound that melted at 151–152° and showed no depression in melting point on mixing with an authentic sample of the pentamethyl ether of quercetin. The above observations prove that the aglycone is a monomethyl ether of quercetin. The possibility of the aglycone having a methoxyl group at C-5 is ruled out as it does not show fluorescence in acetic anhydride.[9] A comparison of the melting points of the aglycone and its acetate with those of known 7-, 5-, 3′-, and 4′-monomethyl quercetins suggested its identity with isorhamnetin. This was confirmed by a comparison of ultraviolet and infrared spectra, by chromatography and R_f values and by a mixed melting point with authentic isorhamnetin.

The glycosides, m.p. 165–167° and 208–210°, give positive[6] tests with magnesium and hydrochloric acid and sodium amalgam followed by acidification, indicating thereby the flavanone or flavonol nature (with C-3 blocked)[10] of the glycosides. The appearance of a yellow color with Wilson boric acid reagent[7] eliminates the possibility of the glycosides belonging to flavanone class. On the basis of the above color reactions both the glycosides have been considered as flavonol glycosides. Both of them

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