Steroids XXVII. Synthesis and Antimicrobial Properties of 4,6β-Dimethyl-4-aza-5α-cholestane

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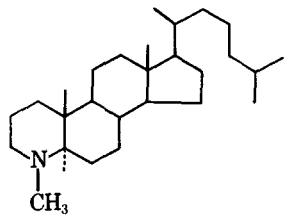
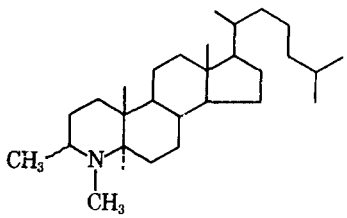
6β-Methyl-4-aza-5α-cholestane (VII) and 4,6β-dimethyl-4-aza-5α-cholestane (VIII) were synthesized from 6α-methyl-4-cholesten-3-one (III) *via* a sequence of reactions which included (*a*) ozonization of III to obtain 6α-methyl-3,5-seco-4-norcholestan-5-on-3-oic acid (IV), (*b*) condensation of IV with ammonium hydroxide to yield 6-methyl-4-aza-5-cholesten-3-one (V), (*c*) hydrogenation of V to 6β-methyl-4-aza-5α-cholestan-3-one (VI), (*d*) lithium aluminum hydride reduction of VI to VII, and (*e*) a Leuckhart methylation of VII to obtain VIII. Compounds VII and VIII possess antibacterial and antifungal properties, but are less active than 4-methyl-4-aza-5α-cholestane (I), a homolog without a 6β-methyl substituent.

ANTIMICROBIAL steroids have been the subject of earlier reports from this laboratory (1–4). The most active of these steroids were derivatives of 4-aza-5α-cholestane, *e.g.*, ND-502, 4-methyl-4-aza-5α-cholestane (I) (4, 5), and ND-307, 3ξ,4-dimethyl-4-aza-5α-cholestane (II) (1–5). These steroids were discovered to be bactericidal *in vitro* against most Gram-positive bacteria and fungicidal against most molds and yeasts. ND-307 was more active than ND-502 against over 30 organisms with which they were compared. The methyl group at position 3, for reasons not known at this time, increased activity. This investigation was undertaken for the purpose of determining the effect of a 6β-methyl group on antimicrobial activity.



ND–502
I



ND–307
II

6α-Methyl-4-cholesten-3-one (III) (6, 7), the intermediate used for these syntheses, was prepared from cholesterol by the procedure described by Turner (7). 6α-Methyl-3,5-seco-4-norcholestan-5-on-3-oic acid (IV) was obtained in an 80% yield by treatment of III with ozone at 0°. The reaction of IV with ammonium hydroxide at 160° in a pressure vessel gave 6-methyl-4-aza-5-cholesten-3-one (V) in 88% yield. The absorption maximum (239 mμ) of this enamine lactam (V) had undergone a bathochromic shift of 6 mμ from that of 4-aza-5-cholesten-3-one (8) as a result of the introduction of the methyl group at position 6.

Hydrogenation of V in glacial acetic acid solution with a platinum catalyst gave a single product, identified as 6β-methyl-4-aza-5α-cholestan-3-one (VI). Hydrogenation of steroidal-5-enes is known to be stereospecific with hydrogen being introduced from the α-face (9). The sterospecificity of the hydrogenation of a related enamine lactam, 4-methyl-4-aza-5-cholesten-3-one (10), to a 5α-isomer has been demonstrated (11). The configuration of the methyl group at position 6 was confirmed by a comparison of the NMR spectra[1] of VI with that of 4-methyl-4-aza-5α-cholestane (10). C-18 absorption shifted by −0.03 p.p.m. (9.28 to 9.25 ν) and C-19 absorption shifted by −0.10 p.p.m. (9.14 to 9.04 ν). A 6β-methyl group in the 5α-series has been reported to cause shifts in C-18 of about −0.02 p.p.m. and in C-19 of about −0.07 p.p.m. (12). The 6α-methyl group in the 5α-series is reported to have no effect upon C-18 and only a slight effect upon C-19. The effect of 6β-groups upon the angular methyl groups is attributed to their diaxial relationships.

The reduced lactam (VI) was treated with lithium aluminum hydride to obtain 6β-methyl-4-aza-5α-cholestane (VII) in 76% yield. Steroidal bases, such as VII, are often low melting and difficult to obtain in crystalline form. Our experience with a variety of such bases indicates that acetonitrile is a solvent of choice for crystallization. 4,6β-Dimethyl-4-aza-5α-cholestane (VIII) was prepared from VII in 45% yield by the Leuckhart methylation using formalin and formic acid.

Compounds VII and VIII were compared with ND-502 (I) and ND-307 (II) for antimicrobial

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[1] Spectra were obtained with a Varian model A-60 NMR spectrometer using deuterochloroform solutions containing tetramethylsilane as an internal reference by Dr. Bernard Grabowski, School of Pharmacy, University of Missouri, using equipment at Midwest Research Institute.

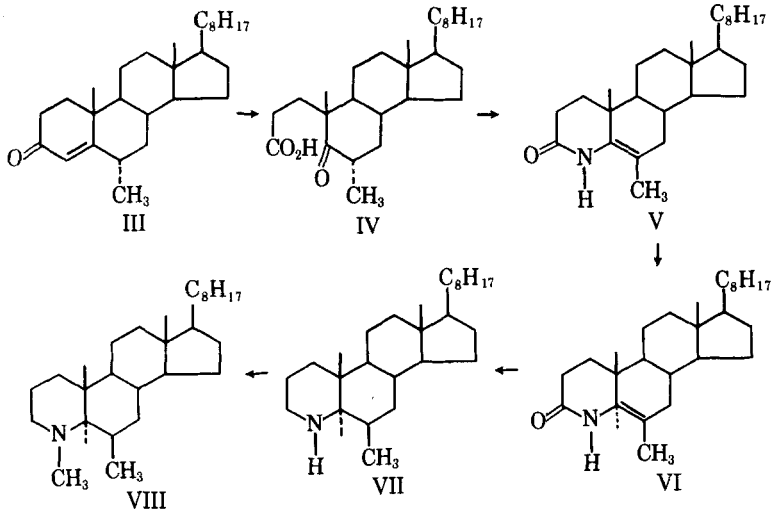

TABLE I.—MINIMUM INHIBITORY CONCENTRATIONS OF STEROIDS

Microorganism	Steroids[a] VII	VIII	ND-502	ND-307
Bacillus cereus	100	[c]	12.5	[b]
Gaffkya tetragena	12.5	[c]	1.5	[b]
Geotrichum candidum	[c]	12.5	[b]	1.5
Candida albicans	[c]	25	[b]	6.1
Saccharomyces cerevisiae	25	50	6.2	3.1

[a] All values are expressed in mcg./ml. [b] Not tested. [c] No inhibition.

activity against a few microorganisms using a serial dilution method previously reported (1–4). Although VII and VIII exhibited activity in these preliminary studies, they were much less active than ND-502 and ND-307. It appears that the 6β-methyl group decreases the antimicrobial activity of 4-azasteroids. (Table I.)

EXPERIMENTAL[2]

6α-Methyl-3,5-seco-4-norcholestan-5-on-3-oic Acid (IV).—One gram (2.4 mmoles) of 6α-methyl-4-cholesten-3-one (III) (6, 7) was dissolved in a mixture of 225 ml. of glacial acetic acid and 75 ml. of ethyl acetate. After cooling to 0°, the solution was treated with a 100% excess of ozone produced by a Welsbach T-23 ozone generator. One milliliter of 30% hydrogen peroxide was added to destroy the ozonide. The solvent was removed *in vacuo*. The oily residue was dissolved in diethyl ether and extracted with dilute sodium hydroxide. The aqueous extracts were acidified and extracted with several small portions of diethyl ether. The extracts were combined, dried over sodium sulfate, and concentrated to 2 ml. and diluted with about 30 ml. of *n*-hexane. After several days in the refrigerator, fine white crystals separated. This product was recrystallized from *n*-hexane to obtain 0.86 Gm. (80%) of IV, m.p. 133–134°; $[\alpha]^{25}$ + 40.8°.

Anal.—Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.44; H, 10.82.

6-Methyl-4-aza-5-cholesten-3-one (V).—A solution of 2.15 Gm. (5.40 mmoles) of IV in 100 ml. of concentrated aqueous ammonium hydroxide was heated in a pressure vessel at 160° for 8 hr. After cooling, the reaction was removed and the solid precipitate was filtered, dried, and crystallized from ethanol–acetone to obtain 1.82 Gm. (88%) of V as white needles, m.p. 203–204°; $[\alpha]_D^{25}$ −82°; $\lambda_{max.}^{EtOH}$ 239 mμ (log ε 4.09); $\lambda_{max.}^{CHCl_3}$ 2.95 μ (N—H), 5.96 μ (C=C), and 6.04 μ (C=O).

Anal.—Calcd. for $C_{27}H_{45}NO$: C, 81.14; H, 11.35; N, 3.51. Found: C, 80.97; H, 11.91; N, 3.69.

6β-Methyl-4-aza-5α-cholestan-3-one (VI).—To a solution of 422 mg. of V in 75 ml. of glacial acetic acid was added 170 mg. of platinum dioxide. The mixture was hydrogenated at 85° and 400 lb. pressure for 5 hr. The catalyst was filtered and the solvent removed *in vacuo*. The semisolid residue was crystallized from ethanol to obtain 335 mg. (79%) of VI as white needles, m.p. 235–236.5°; $[\alpha]_D^{25}$ + 57°; $\lambda_{max.}^{EtOH}$ no absorption at 239 mμ; $\lambda_{max.}^{CHCl_3}$ 2.95 μ (N—H) and 6.04 μ (C=O).

Anal.—Calcd. for $C_{27}H_{47}NO$: C, 80.73; H, 11.80; N, 3.49. Found: C, 80.90; H, 11.16; N, 3.56.

6β-Methyl-4-aza-5α-cholestane (VII).—To a solution of 150 mg. of VI in 250 ml. of anhydrous diethyl ether was added, in small portions, 1.0 Gm. of lithium aluminum hydride. The mixture was refluxed with stirring for 8 hr. Excess hydride was destroyed with ether which had been saturated with water. The mixture was filtered and the filtrate dried over sodium sulfate. The oily residue obtained by evaporating the solvent was crystallized from acetonitrile to yield VII as white needles, 110 mg. (76%), m.p. 87–88°; $[\alpha]_D^{25}$ + 90°.

Anal.—Calcd. for $C_{27}H_{49}N$: C, 83.69; H, 12.75; N, 3.61. Found: C, 83.42; H, 12.95; N, 3.86.

4,6β-Dimethyl-4-aza-5α-cholestane (VIII).—To a mixture of 2 ml. of formalin (37%) and 2 ml. of formic acid was added 100 mg. of VII. The mixture was refluxed 6 hr., and the volatile substances were then removed *in vacuo*. The oily residue was crystallized from acetonitrile to obtain VIII

[2] Melting points are uncorrected and were determined on a Thomas-Hoover melting point apparatus. Optical rotations were determined on 1% solutions in $CHCl_3$ at 25°. Ultraviolet spectra were obtained with a Perkin-Elmer Spectracord spectrophotometer using 95% ethanol solutions. Infrared spectra were obtained with a Perkin-Elmer Infracord spectrophotometer. Analyses were obtained from Drs. Weiler and Strauss, Oxford, England.

as white needles, 47 mg. (45%), m.p. 118–118.5°; $[\alpha]_D^{25}$ + 45°.

Anal.—Calcd. for $C_{28}H_{51}N$: C, 83.75; H, 12.80; N, 3.52. Found: C, 83.57; H, 12.72; N, 3.78.

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Method for Evaluating Dissolution Characteristics of Capsules

By M. PAIKOFF and G. DRUMM

A simple device is described which is useful for dissolution determinations of capsule formulations. It may be utilized in a visual inspection for capsule dispersion or disintegration, and also in the usual chemical dissolution rate determination for predicting possible problems of drug availability from capsules.

ON OCCASION it has been found difficult or almost impossible to devise biological assays to prove that the availability of a pure drug substance has not been altered when mixed with various adjuncts in a dosage form for clinical human testing. These adjuncts are included for weight adjustments and/or to allow mechanized filling of the capsule. Sometimes the drug can not be administered to the animal as the intact human dosage form, but the capsule must be opened, the contents mixed with some liquid carrier, and then administered *via* a stomach tube. This is obviously not the same dosage form intended to be administered to humans.

Dissolution rates of tablets may be determined by several procedures (1–5) employing various agitation intensities, dissolution media, and methods of sampling the solution for drug content assay. These procedures are possible because the tablet does not float to the surface of the dissolution media. Capsules do not behave in the same manner but will float to the top of the dissolution medium, unless the capsule is held under the surface. The use of lead shot or other devices to weigh down the capsule often react with the dissolution media and consequently yield nonreproducible results. Schroeter (6) described a very complex automated method of following the dissolution of tablets and capsules employing the U.S.P. disintegration apparatus and basket rack assembly.

The authors have devised a simple laboratory procedure for screening clinical supplies of new drugs which are prepared in the form of capsules.

EXPERIMENTAL

A capsule holder consisting of a two-bladed glass stirrer with an opening for the capsule is employed. The opening for the capsule is at the base of the stirrer between the two glass blades (Fig. 1). Variously sized orifices can be incorporated into the stirrer to accommodate differently sized capsules. The stirrer rod capsule holder is attached to a Heller stirrer model No. GT 21. The capsule, after being inserted into the holder, is located half-way down into the dissolution medium maintained at 37°. The stirring rate is varied easily, and the duration of mixing is most often 60 min. with appropriate time intervals scheduled therein for sampling.

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RESULTS AND DISCUSSION

This apparatus has been employed to accumulate two types of *in vitro* data.

Visual Dispersion or Disintegration.—The capsule containing formulation is checked for visual dispersion in 400 ml. of distilled water at 37° with a stirring rate of 60 r.p.m. During a period of 1 hr. the capsule is periodically inspected visually to determine if the ingredients of the capsule disperse following a lag period of several minutes for the gelatin capsules to dissolve. Dispersion or disintegration of the capsule ingredients usually indicates a satisfactory capsule formulation. However, if the ingredients of the capsule remain intact after the gelatin capsule has all dissolved (Fig. 2) and little or no dispersion of the capsule ingredients

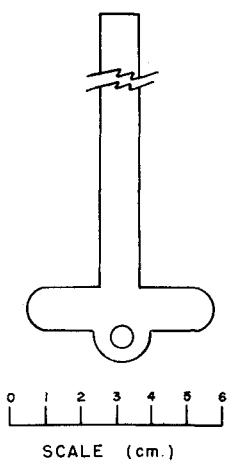

Fig. 1.—Scale drawing of stirrer capsule holder.