0.087 g of a gummy mass. The infrared spectrum of the crude product showed weak carbonyl absorption.

Elution of this mixture through neutral alumina (10 g) with petroleum ether–ethyl ether (9:1) gave 0.012 g of II. Elution with petroleum ether–ethyl ether (8:2) gave 0.067 g of a mixture of two alcohols. Thin layer chromatography (10 cm) of the alcoholic fraction on silica gel G in benzene–ethyl acetate (8:2) showed two components with R_f 0.40 and 0.50 (developed in iodine vapor).

The mixture of alcohols from above (0.067 g) was refluxed in 3 ml of benzene containing a crystal of iodine for 8 hr. The benzene was evaporated *in vacuo* and the reaction mixture was chromatographed on neutral alumina. Elution with petroleum ether gave 0.060 g of IV which crystallized from methanol and had mp 76.5–77.5°. A mixture melting point of IV obtained in this manner and that obtained as described above was undepressed. The infrared spectra of samples of IV prepared by these different routes were identical.

Registry No.—II, 7721-78-0; IV, 7721-79-1.

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Studies on the C-20 Epimers of 20-Hydroxycholesterol[1]

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The evidence presently available[2-4] supports the contention that the biosynthetic pathways through which the steroid hormones are derived from cholesterol involve, at the start of the sequence, 20α-hydroxycholesterol (1,Δ^5-cholestene-3β,20α-diol). Thus, specific hydroxylases present in endocrine tissues catalyze the introduction of a hydroxyl group at C-20 of cholesterol in the α configuration. This paper describes some chemical and spectral (infrared and nmr) characteristics of I and also reports the synthesis and properties of its 20β epimer (V, Δ^5-cholestene-3β,20β-diol).[5]

The 20α epimer was first synthesized by Petrow and Stuart-Webb[6] by the condensation of pregnenolone acetate and isohexylmagnesium bromide. From the Cram rule,[7] the expected product of this reaction is 20α-hydroxycholesterol (for the stereochemical designation, see ref 8 and 9). In our hands, this compound was the only product formed during the condensation, although a careful search was made to isolate the 20β epimer.

The synthesis of 20β-hydroxycholesterol (V) was accomplished using Δ^5-etiocholenic acid chloride as starting material. The acid chloride was treated with diisohexylcadmium, as described by Kurath and Capezzuto[10] to give 21-nor-20-ketocholesterol 3-acetate which was then condensed with methylmagnesium bromide. Preliminary purification by chromatography of the reaction mixture gave evidence of two compounds, in a ratio 10:1, which were best separated by fractional crystallization. The minor compound was identified by its infrared spectrum and melting point as 20α-hydroxycholesterol 3β-acetate (II). Although the infrared spectrum of the major component was similar to that of II, the melting point of the second product (VI) was much lower than that of II. Prediction from the Cram rule leads to the presumption that VI is 3β-acetoxy-Δ^5-cholesten-20β-ol. Saponification yielded V, the infrared spectrum of which is very like that of I. Each of the alcohols, their acetates, benzoates and the Δ^4-3-ketones derived from the alcohols have distinctive melting points (Table I). However, only mixtures of the isomeric alcohols and of their derived Δ^4-3-ketones gave true mixture melting point depressions. In the case of the acetates and the benzoates, the melting points of mixtures of the isomers were unsharp and occurred between those of the individual isomers. The MD values for the isomeric alcohols, their acetates, and benzoates were found to be almost identical for each pair. This is in contrast to the isomeric 22-hydroxycholesterols[11] and the 24-hydroxycholesterols[12] in which the β isomers were found to be more levorotatory than are the α epimers. The rotations of the isomeric Δ^4-3-ketocholesten-20-ols appear to differ significantly.

TABLE I

PHYSICAL CONSTANTS OF 20α- AND 20β-HYDROXYCHOLESTEROLS AND SOME OF THEIR DERIVATIVES

Substituents		20α-Hydroxy series			20β-Hydroxy series		
C_3	C_5	No.	mp, °C	$[\alpha]_D$, deg	No.	mp	$[\alpha]_D$, deg
β-OH	Δ^5	I	136–137	−57	V	115–117	−61
β-OAc	Δ^5	II	156–157	−59	VI	113–114	−47
β-OBz	Δ^5	III	176–178	−38	VII	144–145	−34
Keto	Δ^4	IV	135–137	+65	VIII	129–130	+77

Determination of the infrared spectra of the compounds described in this study revealed that each member of an isomeric pair possesses virtually the same infrared spectrum as its epimer. Moreover, a great similarity was found between the spectrum of cholesterol and those of both 20-OH cholesterols. Thus, the use of infrared spectra as criteria for identification of compounds of this series, at least, is unreliable.

In contrast to the infrared spectra, the nmr spectra of the isomeric 20-hydroxycholesterols and their acetates show very distinct differences in the chemical shifts of the methyl proton resonances. The protons of special interest in this study are the methyl protons on C-18, C-19, C-21, C-26, and C-27. Firm spectral assignments for these protons have been presented[13] for cholesterol. On the basis of these assignments and the relative proton counts under the various peaks, assignment of the features of the spectra of I and V was

(1) This work was supported in part by funds from U. S. Public Health Service Grant No. 00110.

(2) S. Solomon, P. Levitan, and S. Lieberman, *Rev. Can. Biol.*, **15**, 282 (1956).

(3) K. Shmizu, M. Hayano, M. Gut, and R. I. Dorfman, *J. Biol. Chem.*, **236**, 695 (1961).

(4) K. Shmizu, M. Gut, and R. I. Dorfman, *ibid.*, **237**, 699 (1962).

(5) Some of the findings reported in this paper have been presented. See L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 344.

(6) V. Petrow and I. A. Stuart-Webb, *J. Chem. Soc.*, 4675 (1956).

(7) D. J. Cram and F. A. A. Elhafez, *J. Am. Chem. Soc.*, **74**, 5828 (1952).

(8) P. A. Platner, *Helv. Chim. Acta*, **34**, 1693 (1951).

(9) See ref 5, p 339.

(10) P. Kurath and M. Capezzuto, *J. Am. Chem. Soc.*, **78**, 3527 (1956).

(11) K. Tsuda and R. Hayatsu, *ibid.*, **81**, 5987 (1959).

(12) N. Klyne and W. N. Stokes, *J. Chem. Soc.*, 1979 (1954).

(13) "High Resolution N. M. R. Spectra Catalog," Vol. 1, Varian Associates, Inc., Palo Alto, Calif., 1962, Spectrum No. 363.

TABLE II

ASSIGNMENT AND CHEMICAL SHIFTS FOR METHYL-GROUP PROTONS IN CHOLESTEROL, 20α-HYDROXYCHOLESTEROL, AND 20β-HYDROXYCHOLESTEROL

Compd	C-18	—Chemical shifts— C-19	C-21	C-26–C-27
Cholesterol[a]	0.67	1.00	0.90	0.87
Cholesterol	0.66	0.98	0.87	0.84
20α-OH-cholesterol	0.79	0.99	1.17	0.84
20β-OH-cholesterol	0.78	0.94	1.00	0.83

[a] Reference 13.

accomplished. The results are presented in tabular form in Table II and the spectra are shown in Figure 1.

The effect of hydroxyl substitution at C-20 of cholesterol on the high-resolution portion of the nmr spectrum may be divided into three parts. Firstly, the doublet representing the C-21 protons coupled to the proton on C-20 in cholesterol becomes a singlet in the 20-hydroxy compounds. There is negligible coupling with the hydroxyl proton since deuterium substitution had no effect on the spectra. Secondly, the C-18 protons exhibit a downfield shift relative to cholesterol. Within experimental error, this shift is the same in both epimers. Thirdly, the C-21 protons exhibit a downfield shift in both epimers, relative to cholesterol, but the 20α-epimer (I) shows a downfield shift of 0.17 ppm for these protons relative to V. Other effects are probably present, but the spectra are too ill defined to make a study of these possible.

The downfield shifts exhibited by the C-18 and C-21 protons upon hydroxyl substitution at C-20 of cholesterol are expected when account is taken of the electron-withdrawing effect of the hydroxyl group which results in deshielding of the neighboring groups. The effect of particular interest is, however, the difference observed in deshielding at the C-21 position for the two epimers. This effect has been observed before, in the pregnane series[14] where the shift for several 20α isomers was downfield relative to the corresponding 20β compounds. The magnitude of these shifts was comparable to that observed in this study for the 20-hydroxycholesterols. Since polar interactions of a hydroxyl group with a methyl group have never been conclusively shown to exist, such causes for the observed shifts may be ignored. The use of space filling models shows that rotation about the bond forming the intersection of the side chain and the ring system is quite sterically hindered. If rotation is not free about this bond, the C-21 protons of one epimer will reside in an average electronic environment different from that influencing the C-21 protons of the other epimer. Robinson and Hofer[14] have suggested that, in the case of the 20α isomers, the environment is such that a severe nonbonded interaction occurs between the C-12 methylene and the C-21 methyl. Although these environmental effects are probably related to the observed shifts in the nmr spectra, it may not be justified to assign absolute configurations of the products on this basis alone.

Experimental Section[15]

Isohexanol (4-methylpentanol) prepared by the condensation of isoamylmagnesium bromide with formaldehyde[16] was converted to **isohexyl bromide** by published methods.[17]

(14) C. H. Robinson and P. Hofer, *Chem. Ind.* (London), 377 (1966).

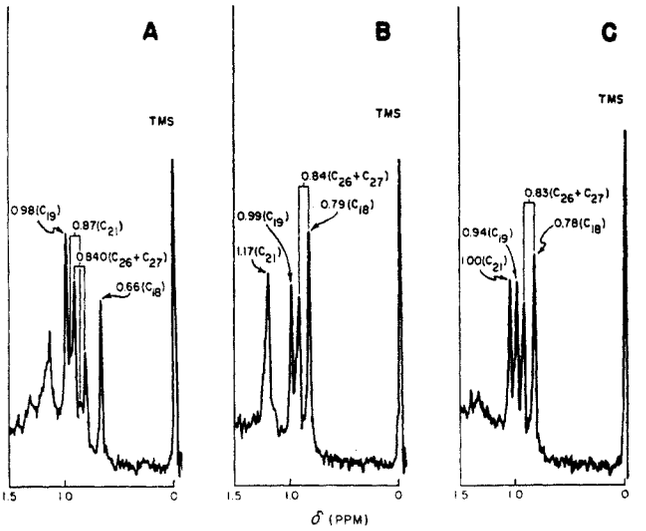

Figure 1.—Nmr spectra: A, cholesterol; B, 20α-hydroxycholesterol; C, 20β-hydroxycholesterol. All spectra were measured in carbon tetrachloride, using tetramethylsilane (TMS) as internal standard.

3β-Acetoxy-Δ⁵-cholesten-20α-ol (II).—This was synthesized by the Grignard reaction between isohexylmagnesium bromide and pregnenolone acetate as described by Petrow and Stuart-Webb.[6] The yield was 48%, mp 156–157°, $[\alpha]^{31}D$ −58.6 ± 1° (36.8 mg in 2 ml of chloroform); lit.[6] mp 155–156°, $[\alpha]D$ −58°.

Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.38; H, 10.69.

20α-Hydroxycholesterol (I).—Compound II (1.05 g) was saponified in 100 ml of methanolic potassium hydroxide (5%) overnight at room temperature. The excess alkali was neutralized by acetic acid, the methanol was removed *in vacuo* and the resulting crystalline material (1 g) was collected by filtration. The crystals were washed with water, dried *in vacuo*, and crystallized from acetone–methanol to give 700 mg (73.5%) of product which, after drying in a pistol, melted at 136–137°, $[\alpha]^{25}D$ −57.7 ± 2° (23.9 mg in 2 ml of chloroform); lit.[6] mp 123–125°, $[\alpha]^{22}D$ −52°.

3β-Benzoyloxy-Δ⁵-cholesten-20α-ol (III).—Compound I (50 mg) was benzoylated in 0.3 ml of pyridine and 0.05 ml of benzoyl chloride at room temperature for 72 hr. The reaction mixture was poured into ice-cold 5% hydrochloric acid and the insoluble product was extracted with methylene chloride. The extract was washed with water, dried over sodium sulfate, and concentrated to an oil *in vacuo*. The oil was dissolved in ligroin–benzene (1:1) and the solution was percolated through 2 g of alumina (Harshaw, washed). The eluted material was crystallized three times from methanol to give 36 mg (57%) of crystals, mp 176–178°, $[\alpha]^{25}D$ −37.5 ± 3° (29.3 mg in 2 ml of chloroform).

Anal. Calcd for $C_{34}H_{50}O_3$: C, 80.58; H, 9.94. Found: C, 80.62; H, 9.77.

20α-Hydroxy-Δ⁴-cholesten-3-one (IV).—Compound I (500 mg) was dissolved in 30 ml of acetone. The solution was cooled to 20° and 0.9 ml of Jones solution (10.3 g of chromic oxide in 30 ml of water and 8.7 ml of concentrated sulfuric acid) was added within 30 sec. After addition of the oxidizing agent, the mixture was stirred for 1 min and then methanol was added to destroy the excess chromic oxide. Water was added and the

(15) The nuclear magnetic resonance (nmr) spectra were taken with a Varian Associates, Inc., DA-60 high-resolution spectrometer. The resolution was maintained at about 0.3 Hz and, since the instrument was field-frequency locked, the spectra were displayed on precalibrated charts. Carbon tetrachloride was used as the solvent with tetramethylsilane (TMS) as an internal nmr standard. The solutions were approximately 1% (w/v). The temperature of the samples, while in the spectrometer probe, was 26°. Infrared spectra were determined on a Perkin-Elmer 221 spectrophotometer. Depending on the solubility of the compounds, spectra were determined using either 5% (w/v) solutions in CS_2 or micro-KBr disks. In the latter cases, the spectra were obtained with the aid of a beam condenser unit. Optical rotations were measured in chloroform solution, in a 1-dm tube using a 0.1–0.2% (w/v) solution.

(16) H. M. E. Cardwell, J. W. Cornforth, S. R. Duff, H. Holtermann, and R. Robinson, *J. Chem. Soc.*, 361 (1953).

(17) S. Sabetay and J. Bleger, *Bull. Soc. Chim. France*, **47**, 885 (1930).

solution was extracted with ether. The extract was washed to neutrality, dried over sodium sulfate and concentrated *in vacuo*. The residual oil (495 mg) was saponified in 30 ml of methanol to which 2 ml of 10% methanolic potassium hydroxide were added. After 15 hr at room temperature, the excess alkali was neutralized with acetic acid and the solution was diluted with water and extracted with ether. The ether solution was washed to neutrality, dried over sodium sulfate, and evaporated to yield a semicrystalline solid (487 mg). This product was chromatographed on 40 g of alumina using 200-ml fractions of benzene as eluent. Fractions 2–6 gave 300 mg of crystalline material, which was recrystallized several times from methylene chloride–chloroform to give IV: mp 135–137°, $[\alpha]^{24}D$ +65.2 ± 1° (28.2 mg in 2 ml of chloroform), $\lambda_{max}^{alcohol}$ 242 mμ (log ε 4.2); lit.[6] mp 137–138°, $[\alpha]D$ +66°.

Anal. Calcd for $C_{27}H_{44}O_2$: C, 80.93; H, 11.03. Found: C, 80.98; H, 11.19.

3β-Acetoxy-Δ⁵-etiocholenic acid was prepared from 3β,21-diacetoxy-Δ⁵-pregnen-20-one by periodate oxidation, using the procedure previously described:[18] mp 237–241°; lit.[18] 241–242°.

3β-Acetoxy-Δ⁵-etiocholenic acid chloride was prepared from 3β-acetoxy-Δ⁵-etiocholenic acid by the method of Steiger and Reichstein:[19] mp 157–159° and 300–310°; lit.[19] mp 331–332°.

21-Nor-20-ketocholesterol 3-acetate was prepared by treating 3β-acetoxy-Δ⁵-etiocholenic acid chloride with diisohexylcadmium as described by Kurath and Capezzuto:[10] mp 139–140°; lit.[10] mp 140–142°.

3β-Acetoxy-Δ⁵-cholesten-20β-ol (VI).—Twenty milliliters of a 3 *M* ether solution of methylmagnesium bromide was added dropwise (in an atmosphere of nitrogen) to an ice-cold solution of 3 g of 21-nor-20-ketocholesterol 3-acetate in 100 ml of dry benzene. The mixture was stirred for 1 hr in the cold and then overnight at room temperature. The ether was distilled and the remainder of the solution refluxed at 75° for 3 hr. After cooling, the Grignard complex was decomposed by ice-cold 10% sulfuric acid. The solution was extracted with ethyl acetate and the extract washed, dried over sodium sulfate, and evaporated *in vacuo*. The crude, oily residue (3.12 g) was acetylated in the usual way overnight and the oil obtained (3.22 g) was chromatographed on neutral alumina. The product isolated from the fractions eluted with ligroin B–benzene (6:4) was recrystallized from acetone–methanol to give 1.9 g (63.5%) of VI, mp 107–110°. Several recrystallizations from methanol gave a product of constant melting point (113–114°), $[\alpha]^{25}D$ −47 ± 3° (10.54 mg in 2 ml of chloroform).

Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.49; H, 10.87.

From the mother liquors, a crystalline product (0.6 g) was obtained, mp 124–127°. Fractional crystallization from ether gave two products, one melting at 131–138° and the other at 114–116°. Further crystallization of the higher melting compound yielded 145 mg (4.8%) of crystals, mp 151–155°. The infrared spectrum of this substance was identical with that of II. Further proof of identity was achieved by saponification and benzoylation to III.

20β-Hydroxycholesterol (V).—Compound VI (134 g, mp 111–112°) was saponified with methanolic KOH in the usual manner. The product was crystallized from methanol to afford 1.11 g of crystals, melting at 115–117°, $[\alpha]^{25}D$ −60.5 ± 3° (24.1 mg in 2 ml of chloroform). Admixture with the 20α-hydroxy isomer (I) gave a depression in melting point (97–115°).

3β-Benzoyloxy-Δ⁵-cholesten-20β-ol (VII).—Compound V (200 mg) was benzoylated as described previously. Crystallization of the product from ether–methanol gave needles: mp 144–145°, $[\alpha]^{25}D$ −33.9 ± 1° (20.6 mg in 2 ml of chloroform). Crystallization from acetone–methanol gave a sample consisting of broad plates. There was no depression in melting point on admixture of this compound with 20α-hydroxycholesterol 3β-benzoate (III).

Anal. Calcd for $C_{34}H_{50}O_3$: C, 80.58; H, 9.94. Found: C, 80.47; H, 9.98.

20β-Hydroxy-Δ⁴-cholesten-3-one (VIII).—Compound V (200 mg) was oxidized by Jones solution as described for the 20α-hydroxy isomer. Chromatography of the crude product on alumina gave material which crystallized from acetone–hexane mp 129–130°, $[\alpha]^{24}D$ +76.6° ± 2° (15.65 mg in 2 ml of chloroform), $\lambda_{max}^{alcohol}$ 242 mμ (log ε 4.19). When this compound was mixed with IV, a large depression (110–124°) in melting point resulted.

Anal. Calcd for $C_{27}H_{44}O_2$: C, 80.93; H, 11.03. Found: C, 80.92; H, 11.10.

Registry No.—I, 516-72-3; IV, 384-27-0; VI, 7429-99-4; VII, 7445-08-1; VIII, 7430-00-4; 3β-acetoxy-Δ⁵-etiocholenic acid, 7150-18-7; 21-nor-20-ketocholesterol 3-acetate, 6570-97-4.

(18) P. Hegner and T. Reichstein, *Helv. Chim. Acta*, **24**, 828 (1941).
(19) M. Steiger and T. Reichstein, *ibid.*, **20**, 1164 (1937).

On Cyclic Intermediates in Hydrolytic Reactions. II. Solvent and Salt Effect in the Alkaline Hydrolysis of Dinitro-2-pyridylalanylglycine

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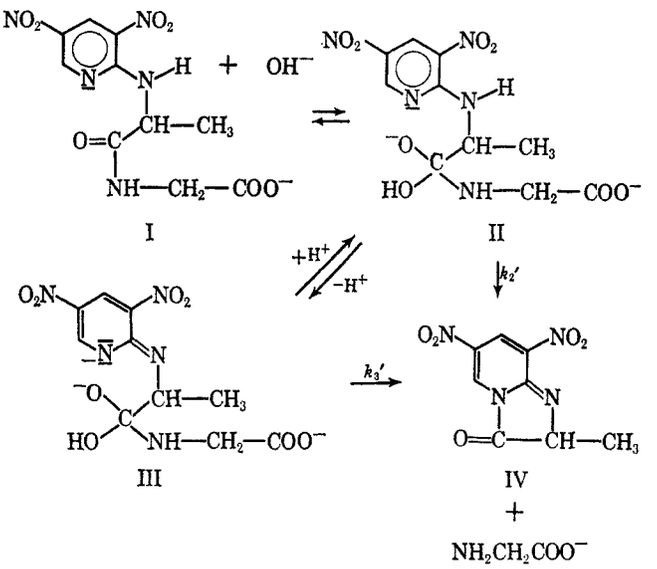
The alkaline hydrolysis of dinitro-2-pyridylalanylglycine (I) has been studied previously;[1] this earlier investigation was concerned mainly with the mechanism of hydrolysis in aqueous solution and the results were consistent with a reaction sequence in which both neutral pyridine nitrogen and pyridyl mesomeric anion give a nucleophilic displacement of glycine anion through neighboring group catalysis (see Scheme I).

SCHEME I



The kinetics of hydrolysis were followed by measurements of the appearance of glycine; moreover it was possible to demonstrate the formation and decomposition of 6,8-dinitroimidazo[1,2a]pyridin-3(2H)-one (IV) in this hydrolysis and to measure its rate of hydrolytic cleavage. The following rate equation was derived on

$$k_1 = k_2[OH^-] + k_3[OH^-]^2 \quad (1)$$

the basis of the proposed reaction scheme where k_1 is the observed first-order rate constant.

(1) A. Signor, L. Biondi, and E. Bordignon, *J. Org. Chem.*, **31**, 1403 (1966).