**8-Hydroxy-9-diethylaminopicrotoxinin 6-Acetate (4c).** To a solution of 0.35 g (0.001 mol) of **2b** in 12 ml of chloroform containing a catalytic amount of *p*-toluenesulfonic acid was added 4 ml of diethylamine. After refluxing for 3 h, the solvent was removed and dilute hydrochloric acid was added. The mixture was extracted with ethyl acetate and the acid solution was evaporated in vacuo to leave a gummy solid: NMR ($CDCl_3$) $\delta$ 0.98 (t, $J$ = 7.0 Hz, $NCH_2CH_3$), 1.33 (s, $C_1$ $CH_3$), 1.40 (s, $C_8$ $CH_3$), 2.10 (s, acetate), 2.09 (d, $J_{11,11}$ = 15 Hz, $C_{11}$ H$\alpha$), 2.40 (s, $C_9H_2$), 2.65 (q, $J$ = 7.0 Hz, $NCH_2CH_3$), 3.3 (m, $C_4$ H), 3.59 (dd, $J_{11,11}$ = 15, $J_{11,12}$ = 3.5 Hz, $C_{11}$ H$\beta$), 3.73–3.87 (m, $C_5$ H, $C_8$ H), 4.93–5.13 (m, $C_2$ H, $C_3$H); ir (KBr) 3450 (OH), 1790 ($\gamma$-lactone), 1730 (acetate), 1260, 1160, 1000 $cm^{-1}$. Anal. Calcd for $C_{21}H_{29}NO_8$·HCl: C, 54.84; H, 6.59; N, 3.04, Cl, 7.71. Found: C, 55.27; H, 6.90; N, 3.17; Cl, 7.80.

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**Registry No.**—**1a,** 17617-45-7; **1b,** 17617-62-8; **2a,** 58281-91-7; **2b,** 58281-92-8; **3,** 58281-93-9; **4a,** 58281-94-0; **4b,** 58281-95-1; **4c** HCl, 58281-96-2; **4e,** 21416-53-5; isopropylamine, 75-31-0; pyrrolidine, 123-75-1; diethylamine, 109-89-7.

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## Conformational Analysis of the 17(20) Bond of 20-Keto Steroids

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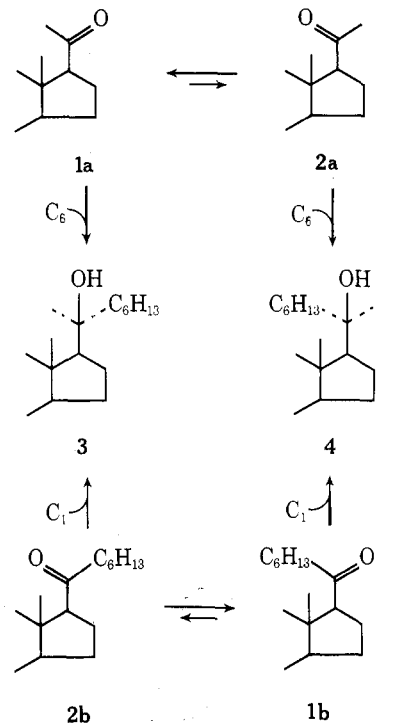
From the reduction of pregnenolone (**1a** and **2a**) and other 20-keto steroids as well as from other data, it has been concluded[1] that rotation about the 17(20) bond can occur and that the preferred conformation at the time of reaction is dependent on certain structural factors. Lithium aluminum hydride reduction of pregnenolone, for instance, yields a 2:1 mixture of the 20-hydroxy epimers.[2,3] As predicted qualitatively by Cram's rule, the dominant epimer is the one derived by $\alpha$-side attack of the reagent on conformer **1a** in which C-21 is cis oriented relative to C-13. Surprisingly, however, the Grignard reaction of the same ketone is reported[4-6] to be completely stereospecific, yielding only the product from conformer **1a,** which led us to a reinvestigation of this problem.

Since the angular methyl group on C-13 should direct attack of the large Grignard reagent to the $\alpha$ face of the steroid, the product composition from the reaction of pregnenolone acetate (**1a** and **2a**) should indicate what the conformational equilibrium is about the 17(20) bond in the ketonic starting material. The $\alpha$-hydroxy product (**3**) must be derived from the cis conformer (**1a**) with the methyl group




toward C-13 and the $\beta$-hydroxy product (**4**) from the trans conformer (**2a**) with the methyl group away from C-13. We have found that both epimers are indeed formed which implies that an equilibrium does exist between both conformers as expected from the work on reductions. Our data also show that conformer **1a** is present in the greater amount at the moment of reaction, since the $\alpha$ epimer (**3**) was present in greater amount in the product mixture, and this is in agreement with deductions based on dipole moment measurements[7] and application of the axial halo ketone rule.[8]

While the cis conformer (**1a**) will yield the 20$\alpha$ epimer (**3** from **1a**) when the incoming group is $C_6$, the 20$\beta$ epimer (**4** from **1b**) should be formed when the incoming group is $C_1$. Furthermore, examination of molecular models reveals that the trans conformer should be destabilized when the alkyl group is increased in size owing to interaction of C-22 with C-16, and the ratio of cis to trans conformer should increase, i.e., the ratio of **1b** to **2b** should be greater than that of **1a** to **2a.** These conclusions require that 20-keto-21-norcholesteryl acetate, which is tantamount to 21-isovalerylpregnenolone acetate, should exist with conformer **1b** being present in larger amount compared to conformer **2b** than is so for conformer **1a** compared to **2a** in the pregnenolone case. The observed facts from the respective Grignard reactions are in agreement, since we find that the 20$\beta$ epimer is formed in much greater amount from 21-isovalerylpregnenolone than is the 20$\alpha$ epimer from pregnenolone. The ratio of 20$\beta$ to 20$\alpha$ epimer in the former case is 10:1.0 while the inverse ratio in the latter case is only 1.7:1.0.

In the NMR spectra of the 20$\alpha$- and 20$\beta$-hydroxycholesterols (**3** and **4,** respectively) the signals for C-18 are exactly the same. The downfield chemical shift compared to cholesterol is $\delta$ 0.19 which agrees with expectation[9] for a 1,3-diaxial relationship between the 20-hydroxy group and C-18. Such a relationship coincides with the conformations of each of the epimers which would result from Grignard reagent attack on the ketone conformers (**3** from **1a** or **2b** and **4** from **1b** or **2a**). The spectrum thus implies conformational preference leading to the structure of the 20-hydroxycholesterol being "frozen" in the conformation existing at the end of the reaction. The 20$\alpha$-hydroxy epimer (**3**)

consequently bears the $C_6$ group on the right, while in the 20β-hydroxy epimer (4) the $C_6$ group must be on the left. Further weight to this conclusion is given by the fact that C-21 would be in a different environment in the two epimers, and the NMR signals for this carbon atom differ (by δ 0.15) in the two cases.

We conclude from these various facts that rotation about the 17(20) bond occurs when C-20 is in the trigonal state. However, when C-20 is tetrahedral and fully substituted, conformational preference is enhanced. It is interesting that in the presence of two substituents on C-20 with the same structure, the addition of restricted rotation could confer asymmetry. This has actually been observed[10] with 20-methyl-20-(2-hydroxyethoxy)pregn-5-ene-3β,17-diol. Despite the fact that C-20 bears two methyl groups, the substance exists as two separable isomers, and they have spectroscopic properties consistent with the ether group lying toward or away from C-13.

## Experimental Section

The NMR spectra were performed in 2% solutions of deuterated chloroform at ambient temperature on a 220-MHz instrument through the services of Morgan-Schaffer of Montreal, Canada, who also supplied the mass spectral data. Melting points were determined on a Kofler hot stage. Gas–liquid chromatography was performed on a column of 1% of nitrile silicone gum (XE-60) on Chromosorb W in a 6-ft U-tube at 235 °C.

**Grignard Reaction with Pregnenolone Acetate (3 and 4 from 1a and 2a).** A benzene solution of pregnenolone acetate was added to 4-methylvalerylmagnesium bromide in ether. After the reaction had subsided the mixture was refluxed for 2 h. The product was acetylated ($Ac_2O$–pyridine, room temperature) and submitted to GLC analysis. Two substances were present with retention times relative to cholesteryl acetate of 2.07 and 1.95 in a ratio of 1.8:1.0 based on peak heights. The NMR spectrum also showed the presence of two substances, one with a C-21 signal at δ 1.28 and the other with the analogous signal at δ 1.13 in a ratio of about 1.6:1.0. The major component was 20α-hydroxycholesteryl acetate (3) and the minor one 20β-hydroxycholesteryl acetate (4) as shown by a comparison of the retention times and NMR values with those of authentic samples which are described in what follows.

The product mixture was crystallized from ethanol, and the precipitate was recrystallized several times, giving 5.1 g (from 14.6 g of pregnenolone acetate) of authentic 20α-hydroxycholesteryl acetate (3) with a retention time relative to cholesteryl acetate of 2.07. The melting point (153–155 °C), NMR spectrum [δ 0.87 (d, $J$ = 6 Hz, 6 H, C-26 and C-27), 0.87 (s, 3 H, C-18), 1.03 (s, 3 H, C-19), and 1.28 (s, 3 H, C-21)], and mass spectrum [$m/e$ 384 ($M^+$ − $CH_3COOH$, 61%), 366 (384 − $H_2O$, 88%), 351 (366 − $CH_3$, 63%), 299 (384 − $C_6H_{13}$, 100%), 281 (299 − $H_2O$, 86%), 256 (87%), 253 (46%), 241 (37%), 228 (61%), 213 (73%), and 211 (52%)] were in agreement with the corresponding values for the product (20α-hydroxycholesteryl acetate) reported earlier.[5,6] The free alcohol derived from hydrolysis (KOH in methanol, 10 min at reflux) of the 20α-hydroxycholesteryl acetate melted at 133–134 °C, δ 0.87 (d, $J$ = 6 Hz, C-26 and C-27), 0.86 (s, C-18), 1.01 (s, C-19), and 1.28 (s, C-21) as previously reported.[5] Although the mother liquor from the first crystallization of the 20α-hydroxycholesteryl acetate was enriched in the β epimer, attempts to obtain the latter pure by concentration and recrystallizations of the succeeding crops of crystals failed. The best material was a mixture in which the β epimer had twice the concentration of the α epimer by GLC analysis.

**Grignard Reaction with 20-Keto-21-norcholesteryl Acetate (3 and 4 from 1b and 2b).** Authentic 20β-hydroxycholesteryl acetate (4) was prepared by the Grignard reaction of 20-keto-21-norcholesteryl acetate with methylmagnesium iodide. This reaction is reported[5] to give both epimers in a ratio of about 1:10 (α to β). The GLC analysis of our reaction product was in agreement with this. After acetylation, chromatography on alumina, and crystallization from methanol the β epimer (530 mg from 1.04 g of ketone) had a retention time relative to cholesteryl acetate of 1.95. Its melting point (110–111 °C), NMR spectrum [δ 0.88 (d, $J$ = 6 Hz, 6 H, C-26 and C-27), 0.87 (s, 3 H, C-18), 1.03 (s, 3 H, C-19), and 1.13 (s, 3 H, C-21)], and mass spectrum [$m/e$ 384 ($M^+$ − $CH_3COOH$, 33%), 366 (384 − $H_2O$, 89%), 351 (366 − $CH_3$, 77%), 299 (384 − $C_6H_{13}$, 40%), 281 (299 − $H_2O$, 100%), 256 (65%), 253 (50%), 241 (32%), 228 (84%), 213 (90%), and 211 (63%)] agreed with the literature.[5,6] The free alcohol melted at 115–117 °C as previously reported.[5] Since the melting point of the β epimer is much lower than that of the α epimer, it is not surprising that the β epimer was missed by earlier workers in the Grignard reaction with pregnenolone. It was easily isolated only when the α epimer was present in very small concentration.

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**Registry No.**—**1a,** 1778-02-5; **1b,** 6570-97-4; **3,** 7484-20-0; **4,** 7429-99-4; 4-methylvaleryl bromide, 58298-91-2.

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# Attempted Synthesis of 2-Methylalanyl-L-prolyl-L-tryptophan. An Unexpected Result

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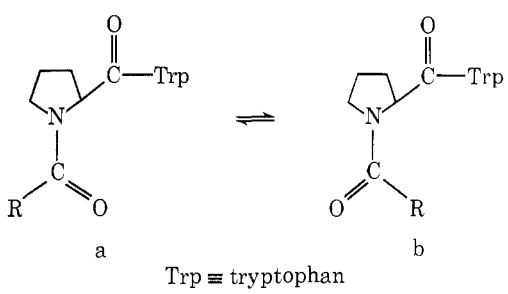
A program in our laboratory required the synthesis of tripeptides containing proline or 4-fluoroproline[1] as the central amino acid residue and an N-terminal group which biased the conformational equilibrium shown in eq 1




(1)

Trp ≡ tryptophan

strongly in favor of one conformer. A recent $^1H$ NMR study has demonstrated that pivaloylproline exists as essentially one rotational isomer.[2] This corresponds to R = $(CH_3)_3C$ in eq 1, and we reasoned that 2-methylalanine [R = $(CH_3)_2CNH_3^+$] as the N-terminal would provide a tripeptide which meets the above specification since the pivaloyl and 2-methylalanyl groups would be expected to be nearly isosteric. Our attempts to synthesize 2-methylalanyl-L-prolyl-L-tryptophan and 2-methylalanyl-4-fluoro-L-prolyl-L-tryptophan by conventional methods did not cleanly afford these tripeptides but culminated, instead, in the results described here.

## Results

The protected tripeptide, carbobenzyloxy-2-methylalanyl-L-prolyl-L-tryptophan (I), was synthesized in ade-