concentrated in vacuo, leaving an oil.

To a well-stirred solution of the above oil in CH_2Cl_2 (15 mL) at room temperature was added pyridinium dichromate (7.0 g, 18.6 mmol). After 5 h, the mixture was filtered through a short plug of silica gel (elution with ether). The eluent was concentrated in vacuo, and the residue was redissolved in benzene and loaded onto a column of basic alumina (30 g). After 48 h, the product was eluted from the column with 50% $CHCl_3$/benzene, volatiles were removed in vacuo, and the residue (565 mg) was chromatographed on silica gel 60 (50 g) eluted with 50% EtOAc/hexanes. The desired decalone **13** was obtained as a colorless oil contaminated with approximately 10% of the cis-fused isomer: yield 485 mg, 2.17 mmol, 83%; $[\alpha]^{24}_D$ +8.46° (*c* 1.75, $CHCl_3$), lit.[7] $[\alpha]^{25}_D$ +7.1° (*c* 2, $CHCl_3$); IR (neat) cm^{-1} 3460, 2939, 2847, 1707, 1466, 1446, 1380, 1307, 1252, 1184, 1153, 1085, 1043, 951, 916, 836, 781; ^1H NMR ($CDCl_3$) δ 0.77 (3, s), 1.19 (3, s), 1.21 (3, s), 1.2–2.0 (12, m), 2.1–2.4 (3, m); ^{13}C NMR ($CDCl_3$) δ (major diastereoisomer) 16.87, 21.39, 21.83, 22.59, 26.61, 27.25, 39.27, 40.26, 40.69, 41.15, 48.35, 57.33, 72.59, 212.92; mass spectrum (70 eV), *m/z* (relative intensity) 224 (0.5), 209 (2), 207 (6), 206 (6), 191 (5), 166 (16), 152 (5), 151 (38), 123 (6), 112 (7), 111 (100), 107 (5), 98 (5), 97 (5), 95 (6), 93 (7), 91 (6), 79 (7), 67 (10), 59 (35); exact mass calcd for $C_{14}H_{24}O_2$ 224.1777, obsd 224.1786.

(+)-β-Eudesmol (1). Ketone **13** (320 mg, 1.43 mmol) was converted to (+)-β-eudesmol (**1**), mp 73–75 °C, lit.[16] mp 80–81 °C (301 mg, 1.36 mmol, 95%) by following the literature procedure:[7] $[\alpha]^{25}_D$ +43.4° (*c* 0.95, $CHCl_3$), lit.[16] $[\alpha]^{27}_D$ +58.0° ($CHCl_3$); IR ($CHCl_3$) cm^{-1} 3605, 3455, 3079, 3020, 2975, 2933, 2867, 2844, 1642, 1455, 1439, 1408, 1378, 1188, 1151, 1122, 1091, 1047, 987, 958, 933, 914, 889, 613; ^1H NMR ($CDCl_3$) δ 0.70 (3, s), 1.20 (6, s), 1.1–2.4 (15, m), 4.44 (1, d, *J* = 1.6 Hz), 4.72 (1, d, *J* = 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 16.28, 22.35, 23.45, 24.99, 27.11, 35.86, 36.86, 41.10, 41.81, 49.42, 49.76, 72.86, 105.30, 151.11; mass spectrum (70 eV), *m/z* (relative intensity) 222 (0.4), 204 (4), 189 (6), 165 (3), 164 (20), 161 (7), 150 (5), 149 (31), 135 (7), 133 (6), 123 (13), 122 (12), 121 (12), 119 (5), 109 (18), 108 (17), 107 (12), 105 (11), 95 (15), 93 (17), 91 (12), 82 (13), 81 (20), 79 (15), 69 (13), 67 (14); exact mass calcd for $C_{15}H_{26}O$ 222.1985, obsd 222.1976.

Stereochemistry of Long-Lasting Opiates. 2. δ-Selective Opiate Antagonists and Their Agonist Analogues†

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We have discovered novel δ-selective opiate antagonists of a non-peptide nature. Our lead compound at the present time is the mixed azine between estrone and naloxone (**16**). In this work we describe syntheses and stereochemical determinations of several antagonist and agonist analogues of **16**, all of which are mixed azines between steroids and opiates. For example, we have prepared the mixed azine between pregnenolone and naltrexone (**17**). A ^{13}C NMR stereochemical analysis showed that **17** was formed as a mixture of two azine isomers: 20(steroid)anti-6(opiate)anti and 20(steroid)anti-6(opiate)syn. The X-ray structural analysis of pregnenolone hydrazone (**8**) (from which **17** was formed) showed 20 anti hydrazone. The X-ray analysis of a single crystal of **17** showed the 20(steroid)anti-6(opiate)anti azine. The C=N—N=C torsion angle was −123°, indicating gauche geometry of the azine bond.

Introduction

Opioid receptors have been pharmacologically classified into several types.[1] Investigation of the physiological significance and molecular properties of different opioid receptor types requires development of type-specific probes. Much information can be obtained by the use of antagonists specifically blocking a certain receptor type. We have synthesized a series of opioid–steroid hybrid azines as potential opioid receptor probes and found them to show long-lasting in vitro activity at the μ binding sites in rat brain membranes.[2-4] Some compounds showed enhanced δ receptor selectivity in vitro.[5,6]

In this study we describe syntheses and detailed stereochemical determinations of several opiate–steroid hybrid azines and steroidal hydrazones they were made from. The latter hydrazones were coupled with opiate ketones oxymorphone, naloxone, or naltrexone (Figure 1). The uncatalyzed coupling between steroidal hydrazones and opiate ketones is quite slow. An attempt to catalyze the latter coupling with catalytic amounts of HCl lead to a rearrangement of the initially formed desired mixed opiate–steroid azine to a mixture of the undesired symmetrical azines, i.e. opiate–opiate and steroid–steroid azines.

Experimental Section

The melting points, elemental analyses, IR, NMR, and mass spectra, and the TLC's were done as described in ref 2. In addition, several ^{13}C and ^1H NMR spectra were obtained on a JEOL FX 200-MHz instrument at the University of Wisconsin—Madison. Thanks are expressed to Dr. Bruce Adams for allowing us to use the latter instrument.

Androstenedione (Δ^4-androstene-3,17-dione, **1**) and pregnenolone (**5**) were obtained from G. D. Searle. Estrone (**3**) was

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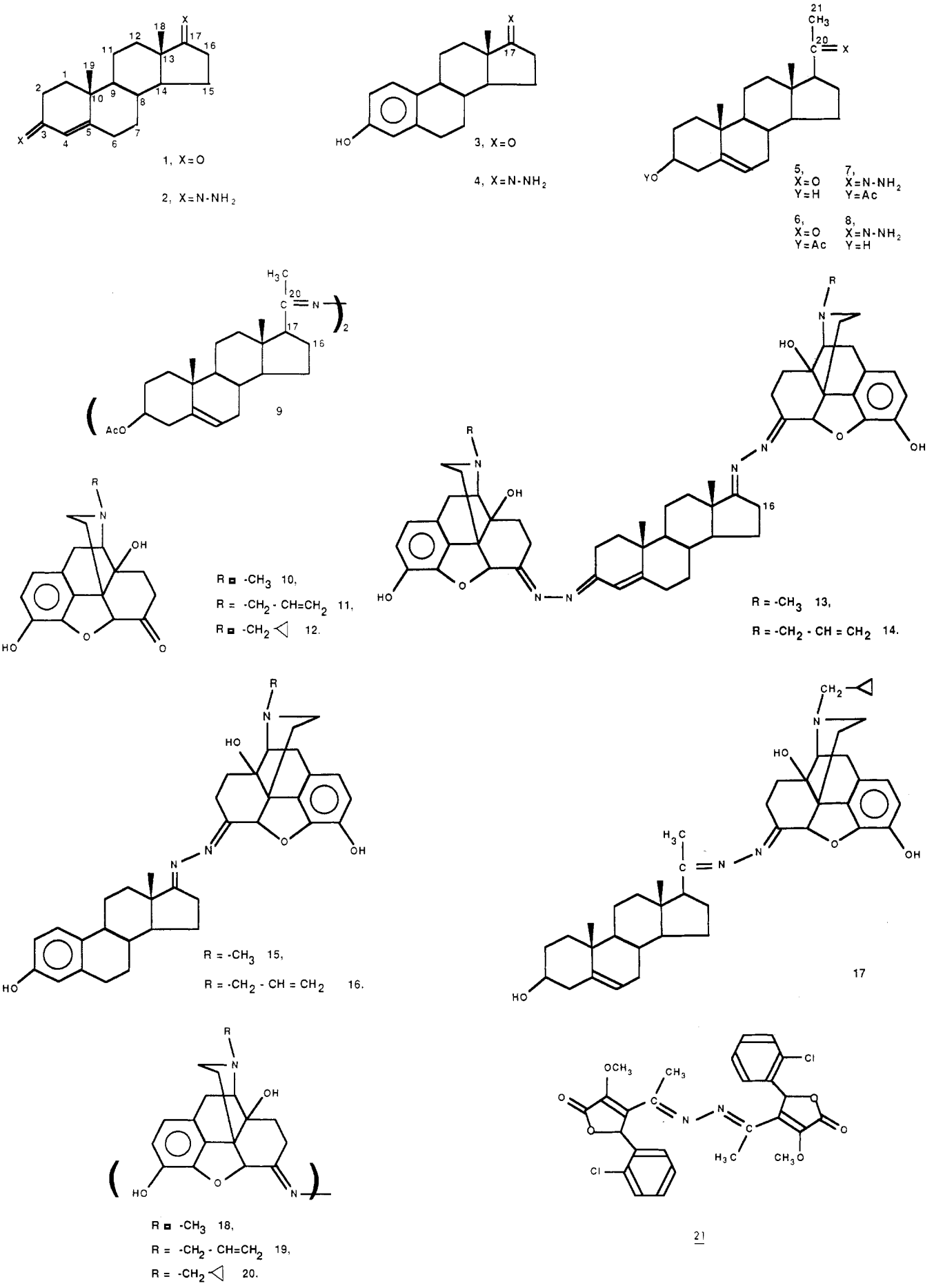

Figure 1. Structures of compounds in this study.

Table I. Some Characteristic ^1H NMR Shifts of the Compounds Studied (ppm)

	steroid moiety							opiate moiety	
compd	CH_3-18	CH_3-19	CH_3-21	H-4	H-3	H-6	3-OAc	H-5	N-CH_3
1[a]	0.926	1.224		5.745					
2[a]	0.887	1.070		5.772					
3[b]	0.80								
4[b]	0.85								
5[a]	0.630	1.009	2.128		3.52	5.345			
6[a]	0.631	1.021	2.128		4.61	5.377	2.038		
7[a]	0.590	1.020	1.761		4.61	5.379	2.035		
9[a]	0.692	1.045	1.762		4.62	5.405	2.057		
8[b]	0.492	0.936	1.639			5.267			
15[a]	0.870							4.945	2.404
16[a]	0.788							5.044	
13[a]	0.861	1.008		5.850				5.023	2.380
	0.878							4.978	
17[b]	0.708	1.071	1.747			4.764		5.000	

[a] $CDCl_3$. [b] Me_2SO-d_6.

obtained from MCB. Oxymorphone (**10**), naloxone (**11**), and naltrexone (**12**) as hydrochloride salts were generously donated by Dr. Alan A. Rubin. These salts were converted into the corresponding free bases as described in ref 2.

The compounds synthesized in this study were obtained in most cases as crude solids. Although these solids were typically essentially pure by NMR, they were extremely difficult to crystallize. A treatment of solids by preparative TLC did not result in the crystalline material. It was concluded that the crystallization is prevented by the presence of isomers (hydrazone or azine) and traces of products of oxidative degradation. Thus, in most cases the elemental analyses were not attempted.

Several solids decomposed before they melted. That was noted as the melting point being higher than the observed decomposition temperature.

Δ^4-Androstene-3,17-dione Dihydrazone (2). A solution of androstenedione (**1**, 500 mg; 1.75 mmol) in 7 mL of warm EtOH (100%) was added dropwise to a solution of hydrazine hydrate (MCB, 2.0 mL; 42 mmol) in EtOH (4 mL). The reaction mixture was stirred at room temperature overnight, after which time the reaction was quenched by being poured into ice. A white solid formed, which was removed by filtration, washed with water, and dried to afford 410 mg (74.5%) of **2**. Recrystallization from EtOH–H_2O provided TLC-pure **2**: R_f 0.30 ($CHCl_3$/MeOH/concentrated NH_4OH, 132:12:0.9); mp >170 °C dec; IR (Nujol) ν 3370, 3210 (NH_2), 1640 (C=N), 1595, 1206, 1180, 1140, 1115, 1085, 1062, 1040, 872, 726, 670 cm^{-1}; ^1H NMR see Table I; ^{13}C NMR data for the major isomer ($CDCl_3$) C-1 34.12[a], C-2 24.31[b], C-3 150.44, C-4 121.37, C-5 165.69, C-6 34.81[a], C-7 32.18, C-8 35.25, C-9 53.89, C-10 37.64, C-11 21.08, C-12 31.61, C-13 43.79, C-14 53.26, C-15 18.86, C-16 23.32[b], C-17 151.17, C-18 16.92, C-19 17.80 (the shifts with the same superscript may be interchanged), the minor isomer C-3 149.28, C-4 109.76 (14.5%); MS (EI), m/e 314 (parent peak). Anal. Calcd for $C_{19}H_{30}N_4$: C, 72.57; H, 9.62; N, 17.82. Found: C, 72.0; 71.9; H, 9.9, 9.8; N, 17.3, 17.3 (analysis by Dr. G. Heselius, Uppsala University, Sweden).

Estrone Hydrazone (4). The compound **4** was prepared as described in ref 7. Some additional spectral data are given here: ^1H NMR see Table I; ^{13}C NMR ($CDCl_3$ + acetone-d_6) C-1 124.98, C-2 111.66, C-3 153.73, C-4 114.01, C-5 136.32, C-6 29.08, C-7 25.79, C-8 37.25, C-9 42.98, C-10 129.84, C-11 26.01, C-12 33.03, C-13 43.31, C-14 51.20, C-15 25.14, C-16 21.72, C-17 157.76, C-18 15.41. Under the conditions of taking spectra a substantial amount of azine between estrone and acetone-d_6 formed: C-17 173.57, C-18 15.60.

Pregnenolone 3β-Acetate 20-Hydrazone (7). First pregnenolone 3β-acetate (**6**) was formed by treatment of pregnenolone (**5**) with Ac_2O in pyridine: mp 148–149 °C (EtOH) (lit.[8] mp 140–151 °C). The hydrazone **7** was prepared by treatment of **6** with an excess of hydrazine hydrate in EtOH, in a procedure analogous to that in ref 9: mp >220 °C dec; R_f 0.49 (0.86 for **6**) (benzene/ethyl acetate, 7:3); IR (Nujol) ν 3340–3440 (NH_2), 1730 (C=O) (s), 1635 (C=N), 1610, 1368 (s), 1333, 1250 (COC, asymmetric stretch) (s), 1198, 1040 (COC, symmetric stretch) (s), 902, 802, 625 cm^{-1}; ^1H NMR see Table I; ^{13}C NMR shifts for **7** as compared to those for **6** (the latter are in parentheses) ($CDCl_3$) C-1 38.11 (38.00), C-2 27.76 (27.66), C-3 73.90 (73.74), C-4 38.89 (38.71), C-5 139.66 (139.56), C-6 122.49 (122.27), C-7* 32.02 (31.73), C-8* 31.78 (31.53), C-9 50.11 (49.81), C-10 36.64 (36.53), C-11 23.10 (22.74), C-12 24.27 (31.73), C-13 43.85 (43.91), C-14 56.23 (56.75), C-15 21.40 (21.40), C-16 37.02 (36.93), C-17 58.97 (63.61), C-18 13.19 (13.15), C-19 21.04 (20.97), C-20 151.67 (209.45), C-21 15.50 (24.43), acetate-CH_3 19.32 (19.24), acetate CO 170.47 (170.47) (the shifts with the asterisks could be interchanged); MS (EI), m/e 372 (parent peak); MS (CI), m/e 373 (M + 1). For **6**: MS (CI), m/e 359 (M + 1); the EI does not give the parent peak at 358. When crude **7** was recrystallized from EtOH–$CHCl_3$ the azine **9** was obtained as a first crop: mp 238–240 °C dec; R_f 0.90 (benzene/ethyl acetate, 7:3); ^1H NMR see Table I; ^{13}C NMR ($CDCl_3$) C-1 38.16, C-2 27.81, C-3 73.95, C-4 39.01, C-5 139.71, C-6 122.55, C-7* 32.15, C-8* 31.84, C-9 50.15, C-10 36.69, C-11 23.42 C-12 24.36, C-13 43.80, C-14 56.55, C-15 21.41, C-16 37.07, C-17 59.17, C-18 13.56, C-19 21.09, C-20 159.06, C-21 19.18, acetate CH_3 19.33, acetate CO 170.49 (the shifts with the asterisks could be interchanged).

Pregnenolone Hydrazone (8). Pregnenolone hydrazone (**8**) was formed by treatment of pregnenolone (**5**) with an excess of hydrazine hydrate in EtOH, in a procedure analogous to that in ref 9. mp >200 °C dec; R_f 0.27 (benzene/ethyl acetate, 7:3); IR (Nujol) ν 3450, 3390 (NH_2, OH), 1638 (C=N), 1615, 1370, 1350, 1291, 1242, 1202, 1075, 1058 (s), 1043, 1009, 951, 800, 660 cm^{-1}; ^1H NMR see Table I; ^{13}C NMR (Me_2SO-d_6) C-1 38.43, C-3 70.06, C-4 42.22, C-5 141.31, C-6 120.42, C-9 49.83, C-10 36.16, C-11 22.73, C-13 43.13, C-14 55.75, C-16 36.99, C-17 58.35, C-18 13.04, C-19 19.20, C-20 147.28, C-21 15.81, MS (EI), m/e 330 (parent peak); MS (CI), m/e 331 (M + 1).

X-ray Structural Determination of 8. A single crystal of 3β-hydroxy-5-pregnen-20-one hydrazone (**8**) with dimensions 0.30 × 0.34 × 0.38 mm was used for the experimental X-ray measurements that were performed on a Nonius CAD4 diffractometer. The systematic absences in the diffraction pattern were consistent with the monoclinic space group $P2_1$ and the cell constants were determined by a least-squares analysis of the 2θ values for 25 reflections (at 20 °C; λ(Cu K_α) = 1.5418 Å). The crystal data are $C_{21}H_{34}ON_2$, M_r 330.52, a = 6.219 (1), b = 25.198 (3), and c = 6.203 (1) Å, β = 106.826 (8)°, V = 930.5 Å^3, Z = 2, D_x = 1.80 g/cm^3.

Integrated intensities were measured for 1980 independent reflections having $\theta < 77°$ using Cu K_α radiation. Lorentz and polarization corrections [1 + $\cos^2 2\theta$] were applied, and normalized structure factor amplitudes were computed. The structure was then solved by a straightforward application of the MULTAN program.[10] The positional and anisotropic thermal parameters

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for all non-hydrogen atoms were refined by full-matrix least-squares using the 1951 reflections for which the observed intensity was greater than twice the corresponding standard deviation. The hydrogen atoms positions were located in electron density difference maps and they were refined isotropically. Weights used were $1/\sigma_{F^2}$[11] with an instability correction of 0.02. For the observed data, the final reliability index (R) was 0.34 R_w = 0.41, S = 2.0, $(\delta/\sigma)_{max}$ = 0.41, $(\delta/\sigma)_{av}$ = 0.15. Atomic scattering factors were taken from the *International Tables for X-ray Crystallography*[12] for neutral atoms and anomalous-dispersion corrections for non-hydrogen atoms were taken from Cromer and Liberman.[13] All calculations except for MULTAN were performed by using XRAY76.[14]

The final coordinates and isotropic thermal parameters ($\times 10^3$) for all the atoms are given in Tables III and IV of the supplementary material. Tables of bond lengths and angles and molecular packing patterns are available from W.L.D. upon request.

Preparation of the Mixed Opiate–Steroid Azines 13–17: General Method. The mixed azines **15** and **16** were prepared by reacting the free base of the opioid ketone **10** or **11**, respectively, with estrone hydrazone (**4**) in 1:1 molar ratio. An ethanolic solution of the steroidal hydrazone was prepared and the free base of the opioid ketone was added to the solution to carry out the reaction. The mixture was stirred at room temperature for 12–24 h. The progress of the resulting reaction is followed by thin-layer chromatography. After the reaction is substantially complete, the reaction mixture is quenched with water, after which the solid product obtained is recovered by filtration and dried. The mixed azine **17** was similarly prepared by treating the opiate ketone **12** with the steroidal hydrazone **8**. A similar method was used to prepare **13** and **14**, the mixed diazines of the opioid ketone **10** or **11**, respectively, and a steroidal dione, Δ^4-androstene-3,17-dione (**1**), except that in the latter case 2 molar equiv of the opioid ketone are reacted with 1 molar equiv of the corresponding dihydrazone of the steroid (**2**). The yields were 75–85% (average).

The progress of these reactions was followed by TLC by using two different ways to detect spots: observing them under UV light and observing the color development after the TLC plate was sprayed with 50% H_2SO_4 and heated to over 100 °C. Oxymorphone (**10**), naloxone (**11**), and naltrexone (**12**) show up very strongly in the UV while the steroidal hydrazones **1**, **4**, **7**, and **8** show up only very faintly. The mixed opiate–steroid azines formed, **13–17**, show up very strongly in the UV. By using the color development method, one can detect the steroidal hydrazones (or their steroidal precursors) very easily. For opioids, Dragendorff's reagent (Sigma; KI 0.11 M; AcOH 3.5 M; bismuth subnitrate 0.6 nM) was also employed as a spraying agent. This reagent gives orange spot on yellow background in the visible light.

Androstenedione dihydrazone (**2**) is greenish, estrone hydrazone (**4**) gives a red spot, and pregnenolone hydrazone (**8**) or pregnenolone 3β-acetate 20-hydrazone (**7**) are light purple. The mixed azines **13–17** are also colored (a color similar to that of the parent steroidal hydrazone), but the opiate ketones **10**, **11**, and **12** do not show up at all in the color development method.

Mixed Diazine between Androstenedione and Oxymorphone (13). Androstenedione dihydrazone (**2**; 23.6 mg; 0.0751 mmol) and oxymorphone (**10**; 45.5 mg; 2 × 0.0755 mmol) were reacted as described in the General Method (vide supra). The compound **13** was isolated as a yellowish solid, 12 mg (18% yield), as an 86:14 mixture of **13** and **2**, respectively (by ^{1}H NMR): R_f 0.78 ($CHCl_3$/MeOH/concentrated NH_4OH, 132:12:0.9); IR (Nujol) ν 3100–3500 (OH's), 1635 (C=N), 1615, 1505, 1250, 1160, 1115, 1105, 1033, 995, 950, 888, 800, 746, 630 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR ($CDCl_3$) for the opiate unit of **13**, C-1 119.47, C-2 117.70, C-3 139.34, 139.30, C-4 143.73, C-5 88.34, 88.04, C-9 64.84, C-11 124.26, 124.23, C-12 129.95, 129.87, C-13 48.86, C-14 70.44, 70.41, C-16 45.68, C-17 42.79, for the steroid unit of **13**, C-4 121.26, C-9 53.93, C-10 38.08, C-13 44.36, C-14 52.96, C-18 16.66, C-19 17.71.

Naloxone Azine·HCl (19) from Naloxone·HCl (11) and Androstenedione Dihydrazone (2). Naloxone·HCl (**11**; 222 mg; 2 × 0.305 mmol), androstenedione dihydrazone (**2**; 96.9 mg; 0.308 mmol), and EtOH (98%; 3 mL) were stirred at room temperature for 20 min, refluxed for 45 min, and let stand for 2 h 15 min at room temperature. A white precipitate, which started forming during the reflux, was removed by filtration, washed with EtOH, and dried in vacuo. This solid was not soluble in common solvents. Its IR spectrum was almost identical with that of naloxone azine·HCl. Both ^{13}C and ^{1}H NMR spectra of the part of the crude solid that was soluble in D_2O indicated substantially pure naloxone azine·HCl.[2] The crude solid was purified by a preparative TLC using as eluent $CHCl_3$/MeOH/concentrated NH_4OH, 132:12:0.9. The major band visible in UV (254 nm), R_f 0.69, was scraped off and extracted with EtOH. The IR spectrum showed a small peak at 1725 cm^{-1}, indicating a breakdown of the product to naloxone, but otherwise was very similar to the IR of naloxone azine free base. Both ^{1}H and ^{13}C NMR spectra (in acetone-d_6) indicated substantially pure naloxone azine free base.[2]

Bisazine between Androstenedione and Naloxone (14). This experiment was similar to the previous one, except that the reaction mixture was refluxed for shorter time (25 min instead of 45 min). The solid that precipitated out of the reaction mixture was washed with EtOH and dried. It was tentatively assigned structure **14**: R_f 0.60 ($CHCl_3$/MeOH/concentrated NH_4OH, 132:12:0.9); mp >280 °C dec; IR (Nujol) ν 1635 (C=N), 1616 cm^{-1}; MS (EI), parent peak at 933 was not observed, fragmentation peaks were observed at m/e higher than 600; no starting materials were present based on the absence of their parent peaks. The solid was virtually insoluble in common solvents, which precluded an NMR analysis. The solid was hydrolyzed with TFA providing chloroform-soluble material, composed of androstenedione and its di- and monohydrazones, and water-soluble part, composed of naloxone amine salt (by ^{1}H NMR).

Mixed Azine between Estrone and Naloxone (16). Synthesis and structural analyses for **16** were given in ref 2. More spectral data are presented here: IR (Nujol) ν 3100–3450 (OH's), 1630 (C=N), 1615, 1500, 1250 (s), 1158, 1118, 998, 945, 930, 625 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR ($CDCl_3$) shifts for the opiate unit of **16**, C-1 119.76, C-2 118.01, C-3 139.59, C-4 143.70, C-5 87.80, C-6 162.30, C-9 63.38, C-11 124.01, C-12 129.61, C-13 49.96, C-14 70.31, C-16 44.75, C-17 57.64, C-18 137.70, C-19 118.34, for the steroid unit of **16**, C-1 126.29, C-2 113.83, C-3 153.52, C-4 115.90, C-5 135.20, C-8 37.95, C-9 43.68, C-10 131.51, C-13 44.25, C-14 52.12, C-17 175.79, C-18 16.60.

Mixed Azine between Estrone and Oxymorphone (15). The compound **15** was prepared by the General Method described above, by treatment of estrone hydrazone (**4**; 91.5 mg; 0.322 mmol) with oxymorphone free base (**10**; 97.4 mg; 0.323 mmol) in EtOH (5 mL). The product **15** was purified on preparative TLC: R_f 0.70 ($CHCl_3$/MeOH/concentrated NH_4OH, 132:12:0.9); IR (Nujol) ν 3100–3500 (OH's), 1635 (C=N), 1610, 1500, 1245, 1158, 1110, 1065, 995, 945, 886, 810, 785, 745, 630 cm^{-1}; ^{1}H NMR data see Table I; ^{13}C NMR ($CDCl_3$ + Me_2SO-d_6) shifts for the opiate unit of **15**, C-1 118.46, C-2 117.07, C-3 139.15, C-4 143.44, C-5 87.37, C-6 159.23, C-8* 28.95, C-9 64.13, C-11 125.49, C-12 130.21, C-14 69.69, C-15* 28.92 (the shifts of the carbons with asterisks could be interchanged), for the steroidal unit of **15** C-2 112.39, C-3 154.40, C-4 114.74, C-5 134.62, C-10 130.86, C-14 51.80, C-17 173.16, C-18 15.90.

Mixed Azine between Pregnenolone and Naltrexone (17). The compound **17** was prepared by the General Method described above, by treatment of pregnenolone hydrazone (**8**; 151.1 mg; 0.458 mmol) with naltrexone free base (**12**; 155.7 mg; 0.457 mmol) in EtOH (minimum amount). The product **17** was a white solid (238.6 mg; 79.8%) composed of two azine isomers (by ^{13}C NMR). The recrystallization from EtOH–$CHCl_3$ provided TLC-pure needles, R_f 0.81 ($CHCl_3$/MeOH/concentrated NH_4OH, 132:12:0.9), which were a single isomer by ^{13}C NMR, and a single crystal (cube) whose X-ray was taken (vide infra): IR (Nujol) ν 3100–3500 (OH's), 1635 (C=N), 1615, 1510, 1338, 1245, 1195, 1155, 1115, 1055, 1035, 995, 945, 800 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR (Me_2SO-d_6) for the opiate unit of **17**, major isomer, C-1 118.57, C-2 117.00, C-3 139.12, C-4 143.68, C-5 87.56, C-6 159.12, C-9 61.47,

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C-11 123.85, C-12 130.63, C-13 47.89, C-14 69.51, C-16 43.36, C-18 9.17, C-19* 3.70, C-20* 3.47 (the shifts of the carbons with asterisks could be interchanged), for the steroidal unit of **17**, major isomer, C-1 38.23, C-3 69.96, C-4 42.16, C-5 141.25, C-6 120.27, C-9 49.63, C-14 55.85, C-18 13.16, C-19 19.09, C-20 157.24, C-21 18.51; for the minor isomer (peaks found in the spectrum of the crude material containing two isomers), C-5 160.17 (opiate), C-20 156.92 (steroid).

X-ray Structure Determination of the Complex of 17, the Major Isomer, and Chloroform. A single crystal of the mixed azine between naltrexone and pregnenolone/chloroform (1:1) with dimensions 0.3 × 0.3 × 0.3 mm was used for the experimental X-ray measurements, which were performed on a Syntex P_3 diffractometer. The systematic absences in the diffraction pattern were consistent with the orthorhombic space group $P2_12_12_1$ and the cell constants were determined by a least-squares analysis of the 2θ values for 25 reflections (at 20 °C; λ(Mo K_α) = 0.71609 Å). The crystal data are $C_{41}H_{55}N_3O_4 \cdot CHCl_3$, a = 17.057 (2), b = 22.645 (2), and c = 10.493 (1) Å, V = 4052.7Å^3, z = 4, D_x = 1.267 g/cm^3.

Integrated intensities were measured for 3330 independent reflections having $\theta < 24°$ using Mo K_α radiation. Lorentz and polarization corrections [1 + $\cos^2 2\theta$] were applied and normalized structure factor amplitudes were computed. The structure was then solved by a straightforward application of the MULTAN program.[10] The positional and anisotropic thermal parameters for all non-hydrogen atoms were refined by full-matrix least-squares using the 2970 reflections for which the observed intensity was greater than twice the corresponding standard deviation. The hydrogen atom positions were located in electron density difference maps and were not refined. Weights used were $1/\sigma_{F^2}$[11] with an instability correction of 0.05. For the observed data, the final reliability index (R) was 0.092, R_w = 0.110, S = 2.9, $(\delta/\sigma)_{max}$ = 0.6, $(\delta/\sigma)_{av}$ = 0.03. Atomic scattering factors were taken from the *International Tables for X-ray Crystallography*[12] for neutral atoms and anomalous-dispersion corrections for non-hydrogen atoms were taken from Cromer and Liberman.[13] All calculations except for MULTAN were performed using XRAY76.[14]

The final coordinates and isotropic thermal parameters (×10^3) for all the atoms are given in Tables V and VI of the supplementary material. Tables of bond lengths and angles and molecular packing patterns are available from W.L.D. upon request.

Results and Discussion

The mixed steroid–opiate azines were prepared by a noncatalyzed treatment of the equivalent amounts of steroidal hydrazones with the opiate ketones. The reversed method of preparation, i.e., a treatment of opiate hydrazones with the steroidal ketones, was found to be unsatisfactory, since small to moderate amounts of the opiate azines were formed at rates competitive to those of formation of the mixed azines. The opiate azines are ultra long-lasting drugs. Their presence in the reaction mixture containing the desired mixed opiate–steroid azines makes the isolation of the latter without a trace of contamination with the former difficult. Thus, an uncertainty in the biological testings of the opiate–steroid azines would be introduced.

A question was posed if the azines, once formed, would "exchange" with the ketones under both uncatalyzed and acid-catalyzed conditions. If they would, the opiate azines would be obtained from the initially formed mixed opiate–steroid azines. This process could happen in vivo also, thus biasing the biological tests.

In an elegant study using ^{3}H-labeled naloxone, Hahn et al.[15] showed that naloxone azine does not incorporate radioactive naloxone in acidic medium (1% AcOH) over a period of 3 h. Also, ^{3}H-labeled naloxone azine did not decompose to naloxone and naloxone hydrazone under similar conditions, as found by the absence of the radioactivity on the TLC plate at the R_f distances for the latter two compounds.

We have similarly found that pregnenolone 3β-acetate azine did not form a mixed azine with naloxone with or without acid catalyst (1.0 M HCl), during a reaction time of 12 or 24 h.

However, both Hahn's and our experiments involved symmetrical azines, which are known to be thermodynamically stable.[16] A question thus remained about the stability of our mixed opiate–steroid azines, which could rearrange to the thermodynamically more stable symmetrical azines—opiate–opiate and steroid–steroid azines.[16,17]

Rearrangement of an unsymmetrical to a symmetrical azine was observed when androstenedione dihydrazone (**2**) was treated with naloxone (**11**) under acidic conditions and prolonged reflux. Naloxone azine (**19**) formed, indicating that a rearrangement of the initially formed mixed azine between androstenedione and naloxone occurred.

However, the mixed azines, once formed, appeared quite stable both in the solid state and in the solution. For example, the mixed azine between estrone and naloxone, **16**, was a pure compound that did not rearrange in $CDCl_3$ during the several hours of accumulation time in an NMR experiment. The mixed azine between pregnenolone and naltrexone, **17**, was formed as a mixture of anti–anti and anti–syn azines (C-20 steroid anti–C-6 opiate anti, and C-20 steroid anti–C-6 opiate syn) on the basis of a ^{13}C NMR of the crude product. When the latter was recrystallized, a pure azine isomer was obtained, assigned to be anti–anti, on the basis of its ^{13}C NMR spectrum. The X-ray structural analysis of a single crystal of **17**, grown from the mixture of isomers, showed the anti–anti structure, but its ^{13}C NMR could not be obtained. The pure isomer of **17** did not rearrange to the symmetrical azines during the time of an NMR experiment (in Me_2SO-d_6), nor did it give the original mixture of anti–anti and anti–syn isomers. The original mixture thus appears to be formed as a kinetic product, similar to the mixture of anti and syn opiate hydrazones formed by a treatment of the opiate ketones **10**–**12** with hydrazine hydrate (**2**).

In this study the choice of opiate ketones was determined to suite the subsequent biological studies. Oxymorphone (**10**; NR = CH_3) is a potent opiate agonist having analgesic activity ca. 10 times higher than that of morphine. The derivatives of **10** were thus expected to show opiate agonist activity. Naloxone (**11**; NR = allyl) and naltrexone (**12**; NR = cyclopropylmethyl) are opiate antagonists, **12** being longer lasting than **11**. The derivatives of **11** and **12** were expected to show opiate antagonist activity.

Since the azines formed were tested for the opioid activity, it was crucial that they did not contain even traces of the starting opioid ketones since the latter exhibit potent biological activities. Thus, the workup was used that would remove traces of the opiate ketones from the mixed opiate–steroid azines. The reaction mixture containing an ethanolic solution of the mixed azines with possible traces of unreacted opioid ketones and steroidal hydrazones was poured into ice–water. The traces of the opioid ketones stay in the ethanol–water solution, as confirmed by a TLC test. The mixed opiate–steroid azines and unreacted steroidal hydrazones precipitate out of the aqueous solution since they are extremely insoluble in water. The traces of the steroidal hydrazones can be removed from the mixed

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opiate–steroid azines by a recrystallization of the crude solid or the preparative TLC.

It is useful to have relatively inflexible probes for studying the opiate receptor which is presumably a flexible peptide. Thus, the knowledge of stereochemistry of the mixed azines between opiates and steroids is of a great importance for a successful interpretation of their long-lasting and δ-selective receptor activity. These mixed azines have several points of flexibility, those in the opiate unit, steroidal unit, and the azine bond. The stereochemical details of the opiate unit are well-known. Axial–equatorial equilibrium of the substituent on nitrogen was determined via a ^{13}C NMR method.[18,19] The existence of the chair and boat forms of the C ring was established via ^{13}C NMR and X-ray methods (the ring C contains the keto group of e.g. naloxone, **11**).[20,21] Also, the energy of the rotamers around the N–C and C–C bonds of the N-allyl and cyclopropylmethyl was calculated.[22,23] Fine details of the stereochemistry of steroids in relation to their receptor binding and biological activity have already been reported by Duax et al.[24] On the other hand, very little is known about the stereochemistry of the azine bond. Both experimental and theoretical data are scarce. There are surprisingly few azine structures in the crystallographic literature.[25-27] The electron-diffraction[28] and the IR and Raman spectroscopic methods[29] indicated the existence of a stable gauche form (a torsion angle C=N—N=C of approximately 60°) in addition to the more stable trans planar form (180°). The molecular orbital calculations, however, did not reproduce the gauche form as a stable conformer at various ab initio levels.[30,31] Only upon an extensive CI treatment was the gauche form observed.[32]

In this study we describe stereochemical determinations of the mixed opiate–steroid azines and the steroidal hydrazones they were derived from by ^{13}C NMR and X-ray crystallographic methods.

The ^{13}C NMR data for each of the compounds in this study is given in the Experimental Section. In the mixed opiate–steroid azines the carbons from the opiate part of the molecule were assigned by comparison with the previously assigned carbons of the opiate hydrazones[2,33] and azines.[2] The carbons from the steroidal part of the molecule were similarly assigned by comparison with the steroidal hydrazones and azines. First, the carbons of the rings not containing the hydrazone or azine groups were assigned by comparison with the appropriate steroidal basic structures reported in the literature.[34-37] Namely, the analysis of the literature data reveals that the substitution in the outer rings, such as the ring D, influences the shifts in the remaining rings, in this case A, B, and C, surprisingly little. For example, the shifts for the carbons C-1 to C-11 of the rings A, B, and C of cholesterol, which has a long alkyl chain at C-17 (in the ring D), and its 17-keto analogue (5-dehydroisoandrosterone) are very similar (all within less than 1 ppm).[34] The shifts of the carbons in the ring containing the hydrazone or azine groups were assigned on the basis of the expected shifts for the anti and syn isomers of oximes,[38] hydrazones,[2,33,39] and azines.[2] The details for specific compounds are presented below.

^{13}C NMR analysis of androstenedione dihydrazone (**2**) revealed the presence of two hydrazone isomers at C-3 (the anti at 150.44, 85.5%; the syn at 149.28, 14.5%). Only one isomer was observed at C-17 (at 151.17), which was assigned to be anti since the corresponding syn hydrazone appears to be sterically very crowded. Namely, the distance between the syn NH_2 and 12βH is only 1.2 Å (based on Dreiding models). There is no obvious steric preference for the C-3 anti hydrazone over its syn isomer. In the former the shortest nonbonding distance from NH_2 is 1.6 Å (to 2αH) as compared to 1.7 Å in the latter (to 4 H). In the sterically noncrowded 17-anti hydrazone, the comparable distance is 2.1 Å (to 16αH).

Estrone hydrazone (**4**) was formed as a single hydrazone. Only one peak per carbon was observed, including the C-17 hydrazone carbon (at 157.76 ppm). The hydrazone was assigned to be anti, since the syn isomer would be sterically very crowded for the same reason as the 17 syn isomer of **2** (vide supra).

The ^{13}C NMR of pregnenolone 3β-acetate 20-hydrazone (**7**) and pregnenolone hydrazone (**8**) indicated the presence of only one hydrazone isomer at C-20 (151.67 and 147.28 ppm, respectively). An inspection of Dreiding models of **7** or **8** reveals that the syn hydrazone suffers from steric crowding. The hydrazone NH_2 clashes with the angular C-18 methyl group and with the 16αH and 12βH. The anti hydrazone is free from such unfavorable interaction. The only steric crowding of the anti isomer is with the C-21 methyl group, which is cisoidal to the NH_2 of the hydrazone. This would indicate that the anti isomer is sterically favored over the syn one.

The anti stereochemistry of the C-20 hydrazone was confirmed by an X-ray structural determination of pregnenolone hydrazone (**8**). The C-20 anti geometry can be extrapolated to **7**. The ^1H NMR shifts can be also related to the C-20 anti stereochemistry. Namely, the observed ^1H NMR shielding of the C-21 methyl group of **7** and **8** as compared to their keto precursors **6** and **5**, respectively (Table I), is probably due to the neighboring (cis) NH_2

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group of the anti hydrazones.

Pregnenolone 3β-acetate azine (**9**) exhibits a ^{13}C NMR very similar to that of its corresponding hydrazone, **7**. The only substantial differences between the azine **9** and hydrazone **7** are for the C-20, which holds the azine or hydrazone group (159.06 for **9**, 151.67 for **7**), and for the C-21 methyl group. The latter carbon is shifted more downfield in the azine than in the hydrazone (19.18 for **9**, 15.50 for **7**). In both azine and hydrazone the C-21 is shifted substantially more upfield as compared to the corresponding ketone **6** (C-21 for **6** is at 24.43).

The ^{13}C NMR of the crude mixed azine between pregnenolone and naltrexone (**17**) indicated the presence of two sets of peaks presumably for two azine isomers: anti(steroid)–anti(opiate) and anti(steroid)–syn(opiate). This assignment was based on the fact that pregnenolone hydrazone (**8**) is obtained as the anti isomer exclusively (vide supra), but the opiate hydrazone is a kinetic mixture of anti (ca. 80%) and syn (ca. 20%).[2,33] Two opiate azine carbons at 159.12 (major) and 160.17 (minor) and two steroidal azine carbons at 157.24 (major) and 156.92 (minor) were observed. The ^1H NMR showed the opiate H-5 of the major isomer at 5.000 and that of the minor isomer at 5.671 ppm.

The crude **17** was recrystallized, providing ^{13}C NMR pure major isomer, which was assigned the anti–anti structure on the basis of its ^{13}C NMR spectrum. The X-ray structural analysis of a single crystal of **17**, grown out of the mixture of isomers, showed it to be the anti(steroid)–anti(opiate) azine (vide infra).

The mixed azines between estrone and oxymorphone (**15**) and estrone and naloxone (**16**) were isolated as single isomers. One peak per carbon in the ^{13}C NMR and single peaks for the steroidal angular methyl at C-18 and the opiate H-5 in the ^1H NMR were observed. The stereochemistry of the azine bond is assigned to be anti at the steroidal C-17, since the starting estrone hydrazone (**4**) is exclusively anti (vide supra). The anti geometry of the azine at the opiate C-6 is assumed, since the anti product is kinetically favored.[2] However, no confirmation of the azine stereochemistry of **15** and **16** was as yet obtained by the X-ray, due to the difficulty in obtaining single crystals.

The structure of the mixed diazine between androstenedione and oxymorphone (**13**) was determined as it follows. The diazine nature of **13** was unambiguously established by an ^1H NMR analysis. The latter demonstrated the presence of the two opioid units per only one steroid unit. The shifts for the opiate H-5 singlets are different for the two oxymorphone units (5.023 and 4.978 ppm). This indicates sensitivity of the H-5 shifts to the steric environment of the azine group. The two H-5 singlets showed an integration area of 2, as compared to the integration areas of 1 for the H-4 from the androstene unit (5.850 ppm) and 4 for the aromatic area (2 aromatic H's per each of the two opiate units). The nonequivalence of the two opioid units observed in the ^1H NMR (vide supra) is amply supported by the ^{13}C NMR. Namely, most aromatic carbons as well as some saturated carbons of the opioid are not single peaks (e.g., C-3 at 139.34 and 139.30, C-5 at 88.34 and 88.04, etc.). The stereochemistry of the azine bonds in **13** is proposed as follows: Anti C-17 (steroid), due to the C-17 anti geometry of the starting androstenedione dihydrazone (**2**); anti C-3 (steroid), since the major isomer of **2** is anti; anti geometry at C-6 of both opiate units, since the anti product is kinetically favored.[2] Again, no confirmation of the structure was done by the X-ray, due to the difficulty in obtaining single crystals.

Table II. Azine Geometry Observed in X-ray Crystallographic Studies

structure	C=N, Å	N–N, Å	N=C, Å	τ,[a] deg	ref
21 planar	1.296	1.379	1.296		25
21 gauche	1.270	1.376	1.284		25
22	1.265	1.410	1.300		26
22	1.278	1.418	1.267		27
17	1.291	1.411	1.256	−123	this study
8	1.267	1.413		30 (H)	this study
				156 (H)	

[a] Torsion angles C=N–N=C (or H).

As shown above, the ^{13}C NMR is a most valuable tool in determining the presence of azine isomers. However, to learn more about the exact geometry of the azine bond, one needs to turn to the X-ray crystallographic method. The latter method would also give a more detailed picture of the entire molecule, which would be of importance for mapping the opiate receptor.

An X-ray crystallographic determination provides a highly accurate picture of molecular geometry, but the latter is valid for a specific solid-state environment. However, studying the same compound in different crystal forms provides additional information on the molecular flexibility of a compound.[40-42] For most uncharged organic molecules such as steroids, a structure observed in the solid state is at or very near a local minimum energy conformation. If the energy of the global minimum is 2–3 kcal/mol lower than that of any metastable state, it is highly probable that a crystal incorporating the minimum energy conformation will be formed preferentially.

The X-ray structure determinations of **8** and **17** offer insight into the extent to which the steroid, the steroid side chain, the naltrexone molecule, and the azine linkage are flexible. The observed conformations of pregnenolone hydrazone (**8**) and the mixed azine between naltrexone and pregnenolone (**17**) are illustrated in Figure 2.

There is a significant difference in the conformation of the pregnenolone skeleton in the two structures due to the inherent flexibility of the unsaturated B ring. The B ring in pregnenolone hydrazone has a perfect 8α,8β-half chair conformation[40] while that in the naltrexone conjugate has a distorted 9α-sofa conformation. Both of these conformations are readily accessible to 5-ene steroids and the conformation observed in any particular steroid depends upon intermolecular and intramolecular factors.

When the C and D rings of the steroids are superimposed by a least-squares process, the twist about the length of the steroid due to the change in B-ring conformation is apparent in the change in A-ring profile. A 10-deg rotation of the progesterone side chain is associated with the formation of the naltrexone adduct.

The torsion angles defining the conformation of the side chain, C(16)–C(17)–C(20)–N(20) and C(17)–C(20)–N(20)–N are −18.60° and −177.7° in **8** and −29.5° and −172.9° in **17**. The nitrogen substituent at C(20) takes up conformations within the range (0° to −46°) observed for the carbonyl of the progesterone side chain.[41]

The naltrexone conformation is nearly indistinguishable from that of naloxone with the exception of the orientation of the substituent on the tertiary nitrogen. Cyclazocine,[43]

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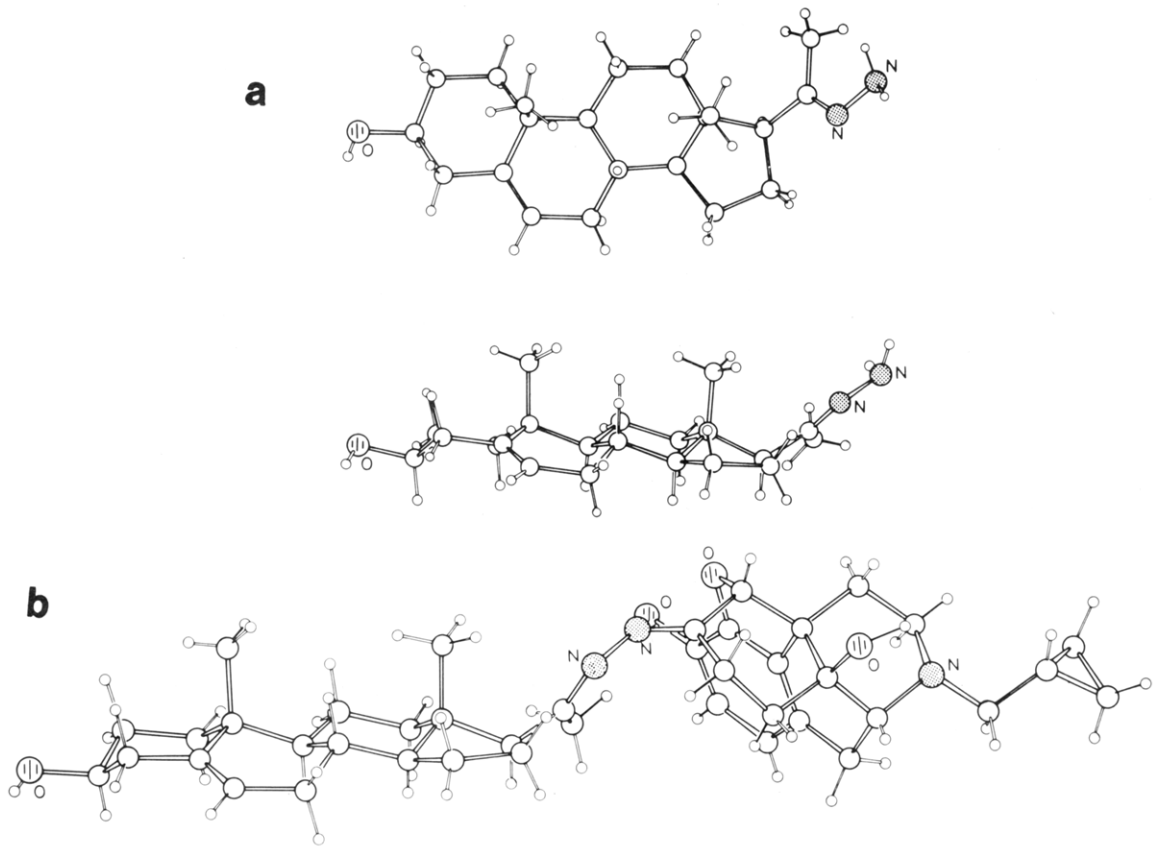

Figure 2. ORTEP structures of the crystallographically observed conformation of (a) 3β-hydroxy-5-pregnen-20-one hydrazone (**8**), two views, and (b) the mixed azine between naltrexone and pregnenolone (**17**).

a morphine analogue with a cyclopropylmethyl containing N-substituent, has a conformation resembling that of naloxone.

There are surprisingly few azine structures in the crystallographic literature. The geometry of the azine in **17** is compared with that observed in two crystal forms of the anti-anti azine **21**[25] and *p*-anisaldehyde azine **22**[26,27] in Table II. The bond lengths are seen to be very similar in the gauche and trans azines. In **17** the N=C bond to the opioid is significantly shorter than the N=C bond to the steroid.

The azine bond has (–) anticlinal stereochemistry with a C=N—N=C torsion angle of –123°. In this structure the gauche conformation (±60°) of the C=N—N=C linkage would be sterically prohibited by the presence of the methyl substituent at C(20). It is not readily apparent why the (–) anticlinal conformation is preferred over a trans conformation and it is impossible to generalize on the basis of the available data on asymmetric azines.

Of interest in this context is the fact that the two hydrogens on the terminal nitrogen (N22) of pregnenolone hydrazone are in (+) synclinal (31°) and (+) anticlinal (156°) conformations.[42] Neither of these hydrogens is involved in hydrogen bonding and consequently the observed conformations are very likely to represent the minimum energy conformation of this side chain. The fact that that neither hydrogen is trans to C=N is consistent with the anticlinal conformation of the azine in the adduct.

The X-ray structure of **17** revealed that the C=N—N=C torsion angle was –123°, indicating gauche stereochemistry of the azine bond. A question arises if the gauche form of **17** is also the most stable form in the solution and at the receptor. The ring C in the opiate moiety of **17** is in a pseudochair form. In cases of some opiates like oxymorphamine, a boat form of this ring was observed[20,21] even though they do not possess the double bond at C-6 like **17**. The presence of the latter double bond in **17** should make the pseudoboat form quite feasible. Again, it would be of interest to determine the solution conformation of the ring C of **17**.

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Supplementary Material Available: Tables III–VI listing fractional positional parameters and isotropic displacement parameters for the non-hydrogen and hydrogen atoms of **8** and **17** (4 pages). Ordering information is given on any current masthead page.