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# SYNTHESIS AND CIRCULAR DICHROISM STUDIES OF STEROIDAL SCHIFF BASES OF BIOGENIC AMINES†

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**Abstract**—A number of imines (Schiff bases) have been prepared from ketosteroids (estrone, estrone-3-methyl ether, androsterone, epiandrosterone, 5α-cholestan-3-one, 6-ketocholestanol and testosterone) and primary amines (phenethylamine, tyramine and hexanamine). The CD spectra of these compounds have been measured in methanol. Except for the testosterone imines, the azomethine $n-\pi^*$ band (~235 nm) exhibited the same sign (but not as high an ellipticity) as the parent ketone's $n-\pi^*$ band (~295 nm). The imines of the estrogens (estrone and estrone 3-methyl ether) gave CD spectra characterized by a negative $^1L_b$ Cotton effect ($[\theta] \simeq -1000$) near 280 nm and a band near 230 nm ($[\theta] \simeq +19{,}000$) which is considered the sum of a positive azomethine $n-\pi^*$ Cotton effect and positive aromatic $^1L_a$ Cotton effect(s). The $^1L_a$ band originating in the amine moiety was prominent in the CD of 5α-cholestan-3-one and 6-ketocholestanol phenethylamine imines. When aromatic Cotton effects originating in the amine moiety were detected, they were of the same sign as the $n-\pi^*$ Cotton effect.

Previous systematic CD studies on chiral imines have concentrated on conjugated imines derived from chiral primary amines and achiral aromatic aldehydes.[1-5] In addition to developing a comprehensive analysis of the conjugated azomethine-benzenoid chromophore these studies have also produced a useful sector rule, The Salicylidenimino Chirality Rule, which correlates amine chirality with imine Cotton effects.[2,3] No comparable studies have been made on the CD of imines derived from chiral ketones and achiral primary amines.

Since ketosteroids comprise a large and well-studied class of chiral ketones, we have prepared a series of imines from ketosteroids and primary amines (Scheme 1). We chose phenethylamine and tyramine imines for study because of potential pharmacological interest in these compounds as neuro-active agents and because in these imines the azomethine and aromatic chromophore of the amine moiety are separated by an ethylene group so that interpretation of the spectra is not further complicated by direct inductive and mesomeric interactions. We sought to ascertain whether the azomethine $n-\pi^*$ band would consistently correlate in sign and intensity with the parent ketone $n-\pi^*$ band, whether the aromatic chromophore in the amine moiety would be sufficiently perturbed by the chiral steroid moiety to give rise to detectable aromatic CD bands and, if so, would these bands correlate with ketosteroid chirality.

Key CD spectra are shown in Figs. 1-5 and comparative spectral values for all imines and parent steroids are listed in Table 1. In the CD spectra of the imines derived from steroids **1-4** the azomethine $n-\pi^*$ Cotton effect appears near 235 nm. Compared to the steroid's C=O band, the imine band has the same sign but narrower band width and lower ellipticity.

In his discussion of the optical activity of azomethines with dissymmetry at carbon,[6] Bonnett notes that, compared to the parent carbonyl compounds, azomethines exhibit Cotton effects of the same sign but at lower wavelength. He also points out that, although the isotropic absorptivity (molecular extinction) is higher for the azomethine, the rotational strength is lower, reflecting the lower magnetic moment of the azomethine $n-\pi^*$ transition.

Any other Cotton effects appearing in the 210-300 nm CD of the imines of steroids **1-4** must originate in the dissymmetrically perturbed aromatic chromophore of the amine moiety. The $^1L_b$ Cotton effect near 270 nm, typically weak for conformationally mobile mono- and di-substituted benzenes, was not observed in the CD of these imines. Compounds **3b** and **4b**, however, exhibited CD bands at 218 nm and 219 nm, respectively (Figs. 2 and 3). Both the narrow bandwidth and the wavelength of this Cotton effect support its assignment to the $^1L_a$ aromatic transition.

No distinct $^1L_a$ band was observed for **1b**, **1c** or **2b**. However, it is evidently present as a weak positive Cotton effect obscured by a much stronger and broader positive azomethine band. This assignment is supported by the higher ellipticity and small hypsochromic shift of the maxima of this composite band for **1c**, the tyramine imine, compared to its phenethylamine analogue, **1b** (Table 1). *Para*-OH substitution on a dissymmetrically-perturbed phenyl chromophore generally enhances the $^1L_a$ and $^1L_b$ Cotton effects.[7] The maxima of this composite band shifts hypsochromically as the lower-wavelength component increases in amplitude.

The CD of testosterone comprises two bands: a negative $n-\pi^*$ (R) band at 317 nm and a strong positive $\pi-\pi^*$ (K) band at 218 nm (Fig. 4). The CD spectra of

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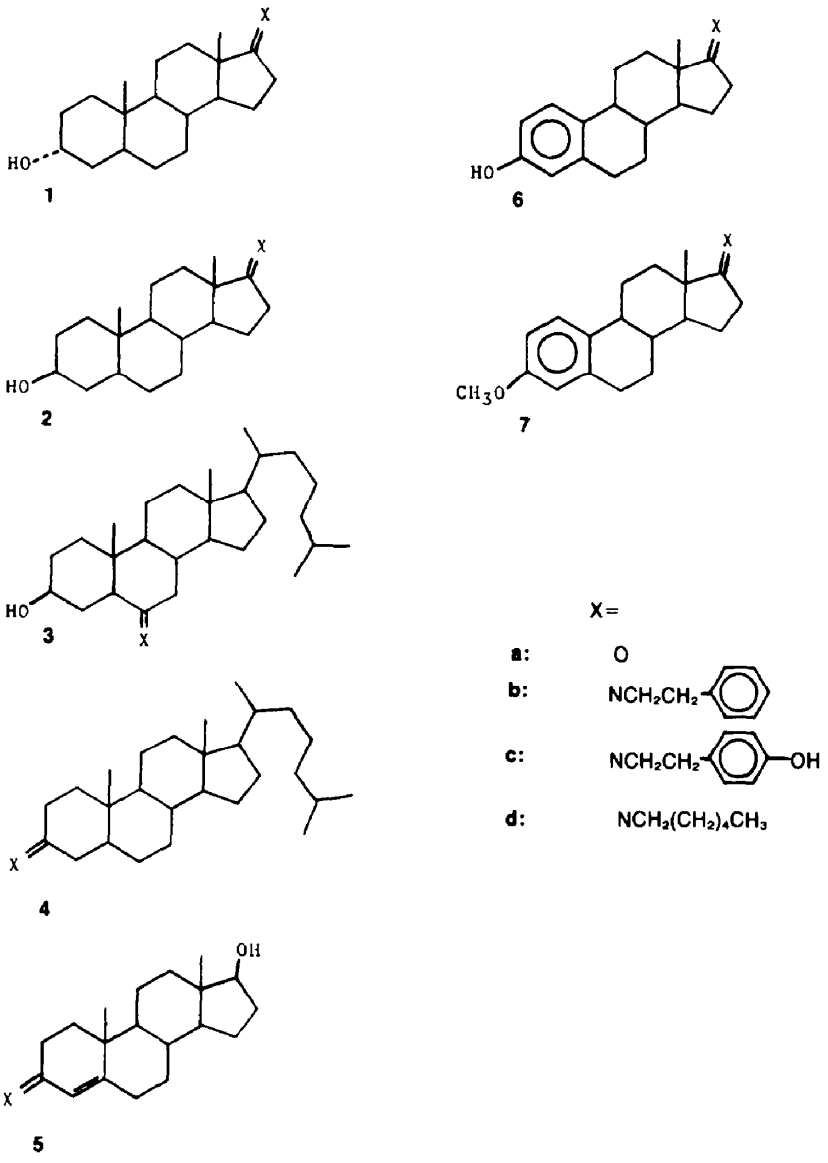


Scheme 1.

imines **5b** and **5c** are positive throughout the observable UV region. An unambiguous rationalization of the spectra is not possible due to the extensive overlap of bands, the number of transitions involved, and the complexity of the inherently dissymmetric $\alpha,\beta$-unsaturated azomethine chromophore. A similarly all positive 220–300 nm CD curve has been reported for the oxime of testosterone[8,9] although, in contrast to the imines, the oxime's ellipticity was greater than that of testosterone itself. The profound increase in ellipticity upon introduction of a *p*-OH (**5c** vs **5b**, Table 1) is too large to be ascribed to the same electronic enhancement of the $^1L_a$ transition seen in other imines studied. More likely there is a charge-transfer or exciton interaction between the aromatic and conjugated azomethine chromophores, where enhancement due to the *p*-OH could be that pronounced.

The CD spectra of the estrogenic imines, **6b**, **6c**, **7b** and **7c** (Fig. 5 and Table 1) is the sum of the azomethine $n-\pi^*$ Cotton effect and the aromatic $\pi-\pi^*$ Cotton effects of the steroid A ring and the amine moiety. The CD spectra of the parent steroids, **6a** and **7a**, consist of two positive bands. The 229 nm band is assigned to the $^1L_a$ transition of the A ring; the 297 nm band is a composite of the positive C=O $n\rightarrow\pi^*$ band superimposed on a weak negative aromatic $^1L_b$ band near 280 nm. In the imine spectra, the $^1L_b$ band at 280 nm is not obscured by the $n-\pi^*$ band. The tyramine derivatives, **6C** and **7c**, produce a slightly stronger band ($[\theta]\simeq-1300$) and the maxima is hypsochromically shifted relative to their phenethylamine analogues, **6b** and **7b** ($[\theta]\simeq-900$), indicating some contribution of the amine moiety to the $^1L_b$ band. However, since the hexanamine imine, **7d**, exhibits a $^1L_b$ Cotton effect of comparable intensity, the 280 nm band must originate primarily in the steroid A ring transition.

The 230 nm band in the CD spectra of the estrogenic imines is a composite of Cotton effects, the sum of

Table 1. CD extrema for steroids and steroid imines[a]

| Compound | Region: <210 nm[b] | Region: 210nm-320nm[c] λ(nm), Bandwidth @ half-height(nm), [θ](deg-cm²/dmole) | | |
|---|---|---|---|---|
| 1a | - | | | 297,35,+13300 |
| 1b | - | | 234,25,+5970 | |
| 1c | - | | 231,24,+6360 | |
| 1e[d] | | | 233, ?,+5200 | |
| 2a | - | | | 297,34,+13600 |
| 2b | - | | 234,24,+6130 | |
| 3a | + | | | 292,33,-5900 |
| 3b | | 218,10,-11100 | 244,28,-4840 | |
| 4a | - | | | 290,37,+6000 |
| 4b | | 219,11,+5510 | 236,∿40,+3750 | |
| 5a | | 218,40,+37600 | | 317,40,-5000 |
| 5b | | | 238,∿40,+18100 | s275,∿30,+5700 |
| 5c | | | 239,48,+28000 | |
| 6a | - | 229,16,+9500 | | 297,31,+12400 |
| 6b | - | | 231,18,+18800 | 279,20,-865 |
| 6c | - | | 230,17,+19500 | 277,22,-1200 |
| 7a | - | 229,16,+9200 | | 296,33,+12200 |
| 7b | - | | 232,18,+17200 | 278,20,-902 |
| 7c | - | | 231,16,+20600 | 276,20,-1380 |
| 7d | - | | 232,20,+18900 | 279,25,-1060 |

[a] 1 millimolar solutions, methanol, 27°

[b] extrema below 210 nm could not be obtained due to the unfavorable anisotropy ratio; where observable, the sign of the next Cotton effect is listed.

[c] ∿ = bandwidth is an estimate (bands are extensively overlapped); s = shoulder

[d] the butanamine imine of androsterone (X = $NCH_2CH_2CH_2CH_3$); [θ] is calculated from the ORD reported in Reference 11 via Kronig Kramers Transform.

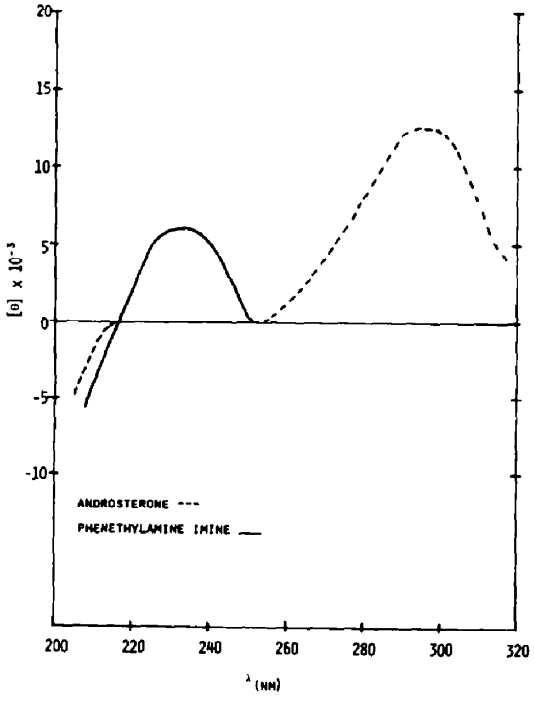


Fig. 1. CD spectra of **1a** and **1b** in methanol.

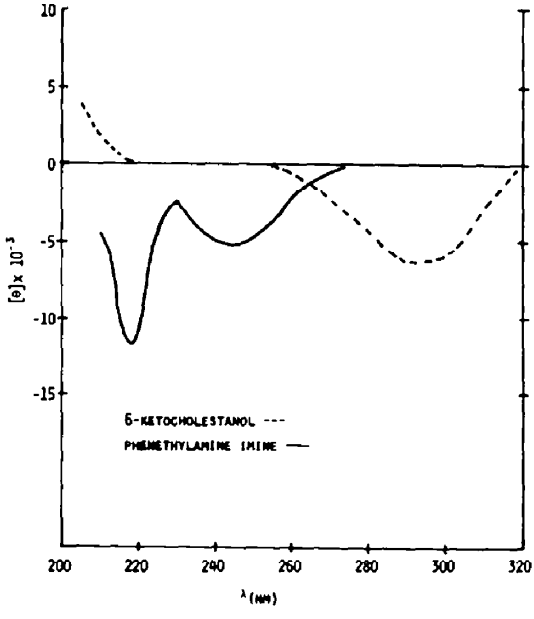


Fig. 2. CD spectra of **3a** and **3b** in methanol.

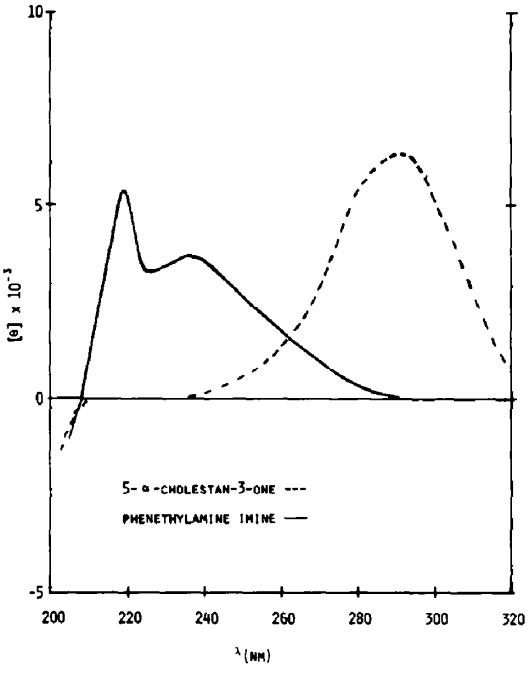


Fig. 3. CD spectra of **4a** and **4b** in methanol.

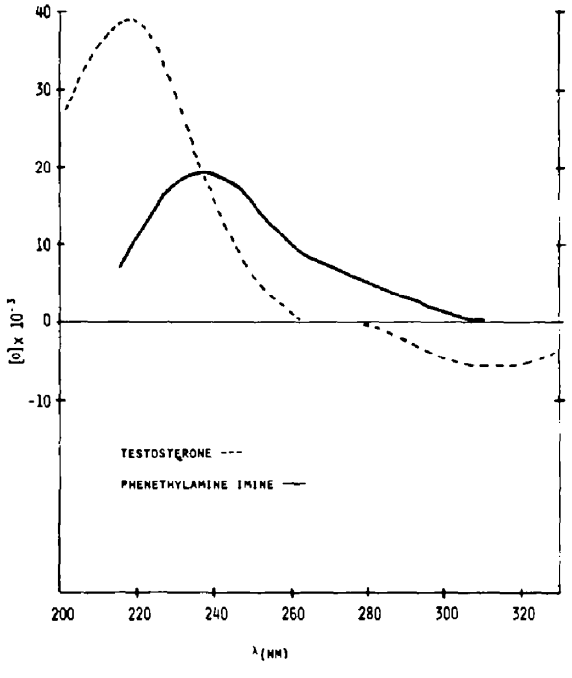


Fig. 4. CD spectra of **5a** and **5b** in methanol.

positive aromatic $^1L_a$ bands and the positive azomethine $n-\pi^*$ band. The changes observed in this band, as the amine moiety is varied, shows that the contribution from the amine moiety's $^1L_a$ transition is minor when compared to the azomethine and A ring $^1L_a$ contributions.

We look forward to continued study of this compound class. We are particularly interested in testing the generality of the apparent correlation found throughout this series, i.e. between the sign of the $^1L_a$ Cotton effect of the amine moiety and the sign of the $n-\pi^*$ Cotton effects of the imine and parent steroid. Also, interpretation of the testosterone imine spectra could be facilitated both by expanding the series and by studying the solvent dependence of the spectra.

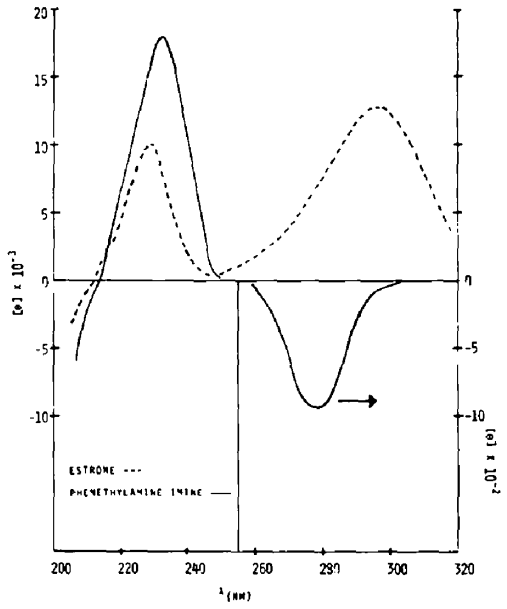


Fig. 5. CD spectra of **6a** and **6b** in methanol.

## EXPERIMENTAL

Steroids and amines were obtained from Sigma Chemical Co., (St. Louis, IL). CD spectra of the imines and steroids were measured on 1 mM solns in MeOH at 27° using a JASCO Model J-20 instrument. CD calibration was conducted with an aqueous soln of d-camphor-10-sulfonic acid ($[\theta]_{290.5} = +7900$).[10] IR spectra were obtained as Nujol mulls or neat (liquid imines) between salt plates on a Perkin–Elmer Model 467 grating spectrophotometer calibrated with polystyrene at 1601 $cm^{-1}$. Mass spectra were obtained on a Finnigan Quadrupole 3100D Spectrometer at 70 eV. NMR spectra were obtained in $CDCl_3$ on a Varian A-60D

Table 2. Thin layer chromatography of imines[a]

| Compound | $R_{f(1)}$ | $R_{f(2)}$ |
|---|---|---|
| 1b | .65 | .85 |
| 1c | .63 | .83 |
| 2b | .57 | .75 |
| 3b | .60 | .84 |
| 4b | .62 | .83 |
| 5b | .59 | .86 |
| 5c | .64 | .89 |
| 6b | .49 | .72 |
| 6c | .54 | .75 |
| 7b | .57 | .78 |
| 7c | .61 | .82 |
| 7d | .68 | .88 |

[a]TLC was conducted, ascending at 28° on Analtech Uniplate RPS brand octadecylsilyl silica, 250 micron plates. $R_{f(1)}$ values are with 99:1 (v/v) methanol/acetic acid as developing solvent; $R_{f(2)}$ values with 4:1 (v/v) ethanol/acetonitrile.

Table 3. Characterization data for steroid imines

| Compound | M.P. (°C) | IR, C=N Stretch ($cm^{-1}$) | Elemental Analysis Calculated C | H | N | Found C | H | N |
|---|---|---|---|---|---|---|---|---|
| 1b | 141-143 | 1669 | 82.39 | 9.99 | 3.56 | 82.39 | 10.02 | 3.59 |
| 1c | 273-276 | 1671 | 79.17 | 9.60 | 3.42 | 79.31 | 9.47 | 3.51 |
| 2b | 103-106 | 1672 | 82.39 | 9.99 | 3.56 | 82.20 | 9.77 | 3.48 |
| 3b | liq. | 1654 | 83.11 | 10.96 | 2.77 | 82.96 | 10.70 | 3.02 |
| 4b | 79-85d | 1658 | 85.82 | 11.32 | 2.86 | 85.64 | 11.25 | 2.76 |
| 5b | liq. | 1630 | 82.81 | 9.52 | 3.58 | 82.50 | 9.48 | 4.22 |
| 5c | 125-128 | 1634 | 79.56 | 9.15 | 3.44 | 79.32 | 9.35 | 3.29 |
| 6b | 166-169 | 1670 | 83.60 | 8.36 | 3.75 | 83.31 | 8.19 | 3.68 |
| 6c | 215-217 | 1667 | 80.17 | 8.02 | 3.60 | 79.97 | 8.05 | 3.47 |
| 7b | 127-129 | 1669 | 83.68 | 8.58 | 3.61 | 83.63 | 8.74 | 3.55 |
| 7c | 191-193 | 1671 | 80.36 | 8.24 | 3.47 | 80.22 | 8.34 | 3.33 |
| 7d | liq. | 1675 | 81.69 | 10.15 | 3.81 | 81.46 | 10.04 | 3.68 |

spectrometer. M.ps were measured on a Laboratory Devices Meltemp and are uncorrected. Elemental analyses were conducted by Galbraith Laboratories, Inc. (Knoxville, TN).

All imines were prepared via co-evaporation of the steroid ketone, the amine, and toluene followed by heating under a $N_2$ purge. In a typical experiment, 0.30 mmole of the ketosteroid and 0.35 mmole of the amine were dissolved or dispersed in 2 ml dry toluene in a 3-in. test tube. After a 10–15 sec $N_2$ purge, the toluene was boiled off as the soln was heated to 150° over a period of 1 hr. The residue was maintained at 150° for 2–3 hr under a slow stream of $N_2$. (With phenethylamine and hexanamine imines, additional amine was added periodically to replace that which evaporated; with hexanamine the reaction temp was 125°.) Progress of the reaction was followed by IR, observing the replacement of the C=O band ~ 1720 $cm^{-1}$ by the C=N band ~ 1660 $cm^{-1}$. Solid imines were purified by recrystallization from abs EtOH. Liquid imines **3b** and **7d** were freed of excess amine by extended $N_2$ purge at 150°, then triturated with diethyl ether and hexane at −40°. The testosterone imine, **5b**, resisted all attempts to rid it completely of phenethylamine. The elemental analysis for **5b** corresponds to 92% imine, 8% amine and CD values were corrected accordingly. Yields for the amine syntheses ranged from 25% to 65%, being lowest for liquid imines.

All of the imines, except **5b**, gave single spots upon reversed phase tlc (RPS Uniplate, Analtech, Inc., Newark, DE) in two different solvent systems: 99:1 (V/V) MeOH/AcOH and 4:1 (v/v) EtOH/MeCN. $R_f$ values are listed in Table 2. NMR spectra and mass spectra of the imines were in agreement with their structural formulas. These selected spectral assignments for **7b** are typical: NMR ($\delta$ from TMS, multiplicity): 0.81, s (C-18, $C\bar{H}_3$); 3.49, t (phenethylic $C\bar{H}_2$); 3.75, s (C-3, $OC\bar{H}_3$) Mass (*m/e*, rel. intensity): 387.18 (parent ion); 296.93 (loss of benzyl). Other data characterizing these imines are presented in Table 3.

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