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CHIROPTICAL PROPERTIES OF N-NITROSOPYRROLIDINES AND N-NITROSAMINO ACIDS[1]

IMPLICATIONS FOR THE NITROSAMINE SECTOR RULE

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Abstract—CD data for a variety of N-nitrosamino acids and N-nitrosopyrrolidines are presented. The effects of nitrosamino group conformation, pyrrolidine ring geometry, different perturbing substituents, and especially intramolecular H-bonding upon the $n \rightarrow \pi^*$ CD band are discussed. Stereochemical conclusions can be made with confidence in many cases, although no sector diagram, as yet published, successfully correlates all the available chiroptical data in this series of compounds. However, a negative CD band due to the $\pi \rightarrow \pi^*$ transition was observed for all N-nitrosamines having the L-proline configuration at C-2, regardless of nitroso group conformation; it is suggested that this band be used whenever possible for stereochemical correlations.

The Cotton effect associated with the $n \rightarrow \pi^*$ transition of the N-nitrosamine chromophore has been used to determine stereochemistry of chiral N-nitrosamines[2] and hence of the derived amines when suitable model compounds were available. In order to permit assignments of absolute configuration to be made directly from the chiroptical data for a single compound without comparison to related compounds of known configuration, Snatzke *et al.* proposed[3] a sector rule for the N-nitrosamine chromophore based upon the nodal properties of the orbitals involved in the high wavelength $n \rightarrow \pi^*$ transition. Somewhat later, Ripperger and Schreiber concluded[4] that application of the nitrosamine sector rule did not always lead to unequivocal configurational assignments, and Gaffield *et al.* suggested[5] that inversion of the sector signs proposed by Snatzke *et al.*[3] was necessary to reconcile CD data for several N-nitrosamino acids. Further chiroptical studies on individual N-nitrosamines were reported to support the original sector assignments of Snatzke *et al.*[3] on one hand[6] and those of Gaffield *et al.*[5] on the other.[7] In 1976, Połoński and Prajer proposed a new lowered symmetry sector rule[8] for the N-nitroso chromophore which appeared to correctly predict the CD properties of both N-nitrosopiperidines and *E* and *Z* forms[9] of N-nitrosamino acids. Ferber and Richardson have reported[10] MO calculations of both sector rules and have concluded that neither rule is generally applicable in predicting the chiroptical properties of N-nitroso compounds. More recently, Ong *et al.* have proposed[11] a new sector rule for the nitrosamine chromophore based also upon the concept of symmetry lowering. Although we have not had an opportunity to study the latter proposal in detail, it appears to have many coincidences with the proposals of Gaffield *et al.*[5] and Połoński and Prajer.[8]

In an effort to reconcile differences among the sometimes conflicting sector rules mentioned above, we have studied the CD properties of two related series of heterocyclic N-nitrosamines of known configuration and conformation. The present paper summarizes our results and interpretations.

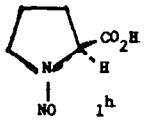
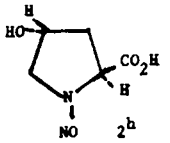
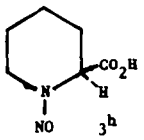
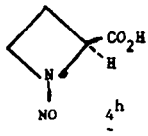
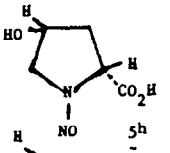
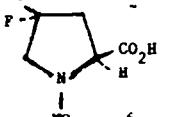
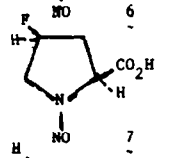
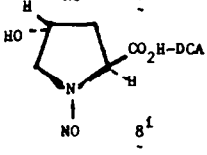
RESULTS

N-Nitrosamino acids. The CD data we have obtained were generally consistent with the sector rules of Snatzke *et al.*[3] as modified by Gaffield *et al.*[5] Table 1 contains CD data on N-nitroso derivatives of L-proline (**1**), *trans*-4-hydroxy-L-proline (**2** and **8**), L-pipecolic acid (**3**), L-azetidine carboxylic acid (**4**), *cis*-4-hydroxy-D-proline (**5**) and *trans* and *cis*-4-fluoro-L-proline (**6** and **7**, respectively).

In aqueous solution, nitrosamino acids **1–3** (predominantly in the *Z*-conformation) gave positive $n \rightarrow \pi^*$ CD bands and **4** (predominantly in the *E*-conformation) gave a negative CD band, each of which decreased in magnitude with increasing time. Liberek *et al.* have shown[9] that pure *E*- and *Z*-forms of **1** give rise to negative and positive CD bands, respectively; the same conclusion held for its monomethylamide. Thus, the change in CD of $1 \rightarrow 4$ with time must primarily reflect equilibration of *Z*- and *E*-conformers, with some additional loss of magnitude possibly due to racemization via carbanion formation at the C-2 position.[12]

The CD curves of the nitroso derivatives (**5** and **2**) of *cis*- and *trans*-4-hydroxyproline were opposite in sign, demonstrating that the C-4 OH group, which has the same absolute configuration in both molecules, has less effect on the CD of these compounds than does the C-2 carboxyl group, which has the opposite configuration in compounds **2** and **5**. N-Nitroso-L-azetidine carboxylic acid (**4**) exhibited negative and positive $n \rightarrow \pi^*$ CD bands in water and pyridine, respectively. This change of sign of CD with solvent may result from the carboxyl group of **4** being in-

Table 1. Circular dichroism of *N*-nitroamino acids $[\theta] \times 10^{-3}(\lambda)$

Nitroamino acid	Solvent (Temp. °C)	Z/E[a]	-N-NO n → π*	-N-NO π → π*	-CO_2H n → π*
 1[h]	pyr (27°)	74/26	+3.16 (362)[b] +1.28 (362)[c]	--	--
	pyr (27°)	80/20	+3.78 (362)[b] +1.34 (362)[c]	--	--
	H_2O (0°)[d]	74/26	+1.23 (342)[b] +0.19 (350)[c]	-25.0 (238)[b] -18.6 (238)[c]	+18.6 (208)[b] +19.0 (206)[c]
	H_2O (0°)[d]	80/20	+1.79 (342)[b] +0.11 (350)[c]	-24.9 (238)[b] -16.3 (238)[c]	+14.7 (207)[b] +15.0 (206)[c]
 2[h]	pyr (27°)	95/5	+4.45 (359)[b] +1.06 (359)[c]	--	--
	H_2O (0°)[d]	95/5	+3.20 (342)[b] +1.21 (345)[c]	-31.6 (238)[b] -22.8 (238)[c]	+14.8 (208)[b] +13.6 (206)[c]
 3[h]	pyr (27°)	64/36	+11.1 (370)[b] +2.35 (370)[c]	--	--
	pyr (27°)	78/22	+11.7 (370)[b] +4.20 (370)[c]	--	--
	pyr (27°)	82/18	+11.0 (370)[b] +3.03 (370)[c]	--	--
	H_2O (0°)[d]	78/22	+5.41 (348)[b] +1.50 (345)[c]	-44.9 (238)[b] -31.9 (238)[c]	+23.5 (195)[e] +20.9 (200)[e]
 4[h]	pyr (0°)[d]	13/87	+0.78 (378)[b] +0.50 (378)[c]	--	--
	H_2O (0°)[d]	13/87	-2.74 (333)[b] -1.18 (333)[c]	-20.3 (240)[b] -19.2 (241)[c]	+25.9 (207)[b] +22.3 (207)[c]
 5[h]	pyr (27°)	75/25	-2.70 (375), -3.00 (362), -2.10 (350)sh[b]	--	--
	MeOH (27°)	--	+0.05 (396), -1.90 (358)[b]	+24.3 (237)[b]	--
	H_2O (27°)	--	-1.90 (348)[b]	+22.8 (237)[b]	-26.9 (208)[b]
 6	pyr (27°)	--[f]	-0.16 (392), +1.90 (374)[b]	--	--
	H_2O (27°)	--[f]	-0.28 (382), +1.90 (344)[b]	-19.9 (237)[b]	--
 7	pyr (27°)	--[f]	+1.20 (384), +0.50 (368), +0.15 (352)[b]	--	--
	H_2O (27°)	--[f]	+0.13 (373), -1.40 (337)[b]	-19.6 (237)[b]	--
 8[i]	H_2O	80/20	+2.94 (345)[b] +1.16 (342)[g]	-33.2 (239)[b]	--

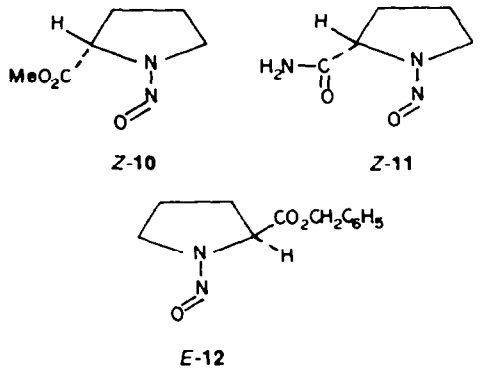
[a] Z/E ratio in the crystalline material as determined by NMR of the freshly dissolved material (Ref. 12).
[b] CD spectra measured immediately after dissolution.
[c] CD spectra measured after standing 24 hr.
[d] Bath temperature measured as 0°, allowing the actual sample temperature to be slightly higher (i.e. not frozen).
[e] Lowest wavelength measured, no maximum observed.
[f] Z/E ratio not determined due to insufficient sample.
[g] CD measured after addition of two moles of HCl to solution.
[h] Prepared according to ref. 12.
[i] Prepared according to ref. 14.

tramolecularly H-bonded in water but with the H-bond broken in pyridine due to ionization of the carboxyl (see below). N-Nitroso-*trans*-4-fluoro-L-proline (**6**) gave a positive $n \rightarrow \pi^*$ CD band in both water and pyridine, similar to **2**, while the *cis*-4-fluoro-L-isomer **7**, structurally analogous to **5**, gave a positive CD band in pyridine but a negative one in water. These observations suggest that a fluoro substituent at C-4 may have a dominant effect on the $n \rightarrow \pi^*$ CD.

In hopes of converting *Z*-**2** into the *E*-isomer, the dicyclohexylamine (DCA) salt of **2** was prepared.[13] NMR showed **2**-DCA also to exist predominantly in the *Z*-form. The CD for this material exhibited a positive $n \rightarrow \pi^*$ CD band similar to that shown by *Z*-**2**. Upon addition of acid to the solution of the DCA salt, the CD was similar to that observed for equilibrated *Z*, *E*-**2**.

N-Nitrosopyrrolidines. Table 2 contains CD data on N-nitroso derivatives of 2(*R*)-methylpyrrolidine (**9**), 2(*S*)-pyrrolidinemethanol (**13**) and the methyl ester (**10**), amide (**11**) and benzyl ester (**12**) of L-proline.

N-Nitrosopyrrolidines **9**–**12** gave positive CD bands at the $n \rightarrow \pi^*$ nitrosamine transition in several solvents, except that **12** gave a negative band in chloroform. Similar to N-nitrosamino acids **1**–**3**, the methyl ester **10** and amide **11** preferred the *Z*-nitroso conformation by a ratio of about 2:1. However, when the carboxyl group was derivatized with a bulky group such as the benzyl ester (**12**) or replaced with a nonpolar substituent such as the methyl group (*cf* **9**), *the E*-nitroso conformer became predominant.



Some interesting solvent effects of possible significance in interpreting the CD spectra of nitrosamines were found for compound **13**. In certain high dielectric constant solvents, including pyridine, dimethylformamide, dimethylsulfoxide, acetone, methanol, 2-chloroethanol and aqueous dimethylamine, two CD bands of opposite sign were observed in the $n \rightarrow \pi^*$ region. The higher wavelength band(s) (approx. 385 nm) was weaker than the negative lower wavelength band(s) (340–380 nm) in every solvent except pyridine. In less polar solvents such as chloroform, carbon tetrachloride, and cyclohexane, **13** showed only negative CD bands at the $n \rightarrow \pi^*$ transition, except in benzene, which behaved like the polar solvents mentioned above. Addition of dimethylsulfoxide (DMSO) to a CCl_4 solution of **13** converted the CD spectrum from one showing only negative bands into one having both positive and negative bands (Fig. 1). This transformation had occurred by the time the solution contained 0.5% DMSO.

Dilute solution ^{1}H NMR spectral data of **13** in CCl_4 and DMSO have indicated the presence of two signals due to the –OH proton (Figs. 2–4). In 75% DMSO/25% CCl_4 (Fig. 2), a triplet due to *E*-OH occurs at 4.92 ppm and another due to *Z*-OH at 4.70 ppm, while in 95% CCl_4/5% C_6D_6 containing 0.033% DMSO (Fig. 3) the relative positions of the *E*- and *Z*-OH signals are reversed, the *E* double doublets appearing at 2.29 ppm and the *Z*-OH signals at 2.51 ppm. Dilute solution spectra of **13** in 95% CCl_4/5% C_6D_6 (Fig. 4) showed only the *Z*-OH signal at 2.29 ppm since the *E*-OH signal was buried (see ^{13}C NMR data in Experimental) under the resonances due to the C-3 and C-4 methylene protons near 2.0 ppm.

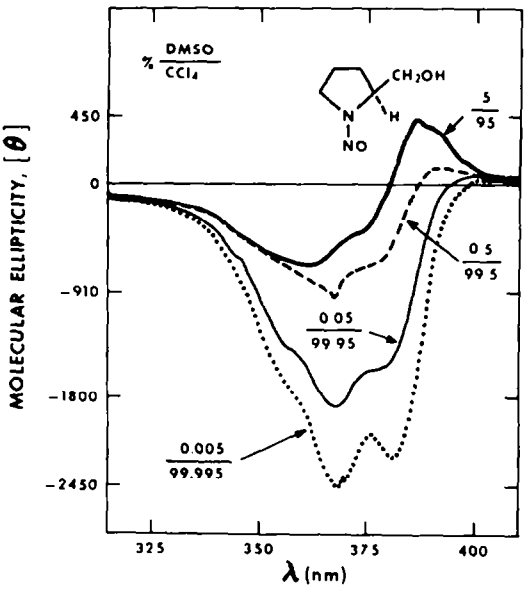


Fig. 1. CD spectra of **13** in CCl_4 upon addition of increments of DMSO.

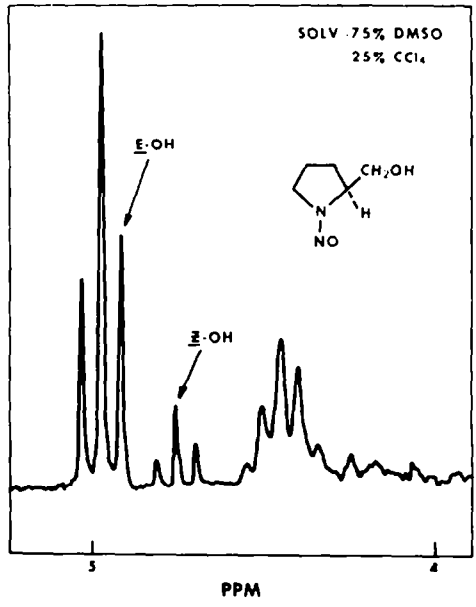
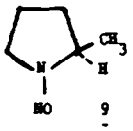
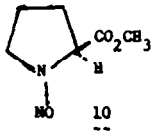
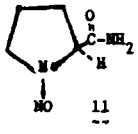
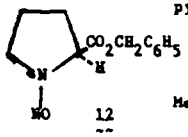
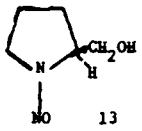


Fig. 2. ^{1}H NMR spectrum of **13** in 75% DMSO/25% CCl_4. Solute concentration is 0.050%.

Table 2. Circular dichroism, $[\theta] \times 10^{-3}(\lambda)$, and ultraviolet spectral data for *N*-nitrosopyrrolidines

Nitrosamine	Solvent	Z/E[a]	N-NO $n \rightarrow \pi^*$	N-NO $\pi \rightarrow \pi^*$	$[\varepsilon]$ (λ)
 9	pyr	14/86	+2.80 (373), +3.00 (364), +1.20 (339)sh	--	110 (376)sh, 140 (364)
	MeOH	--	+0.50 (381)sh, +1.50 (353)	-6.60 (232)	91 (348), 8100 (232)
	C_6H_{12}	--	+2.30 (386), +2.50 (373)	-5.00 (233)	100 (385) 120 (371) 95 (360), 7300 (234)
	$CHCl_3$	--	+2.33 (363)	--	--
 10	pyr	65/35	+2.00 (370), +2.30 (360), +1.90 (349)sh, +0.80(330)sh	--	110 (377)sh, 130 (364)
	MeOH	--	+0.60 (373)sh, +1.10 (359)sh, +1.10 (349)	-23.6 (237)	43 (380)sh, 93 (355), 6740 (235)
 11	pyr	66/34	+1.50 (372), +1.70 (362), +0.60 (332)sh	--	110 (377)sh, 130 (364)
	MeOH	--	-0.09 (389), +0.40 (375)sh, +1.43 (348), +0.63 (324)sh	-26.0 (238)	41 (384)sh, 97 (354), 6350 (236)
 12	pyr	30/70[b]	+2.25 (373), +2.77 (360)	--	110 (377)sh 130 (364)
		37/63[b]	+2.16 (373), +2.82 (360)	--	--
	MeOH	--	+0.30 (374), +0.70 (358), +0.80 (347)	-20.0 (240)	86 (354) 6900 (236)
	$CHCl_3$	50/50	-1.81 (364)	-25.7 (242)	--
 13	pyr	14/86	+0.93 (384), +0.12 (368), -0.24 (359), -0.18 (350)	--	120 (377)sh, 145 (364)
	MeOH	--	+0.21 (387), -1.60 (344)	-6.8 (236)	29 (380)sh, 95 (350), 8100 (234)
	C_6H_6	17/83	+0.13 (393), -0.77 (379), -1.14 (366), -0.93 (354)sh	--	--
	C_6H_{12}	--	-1.51 (385), -1.61 (371), -1.09 (358)	-7.1 (239)	--
	CCl_4	17/83	-2.21 (383), -2.44 (368)	--	107 (367)
	$CHCl_3$	20/80	-3.06 (362)	--	113 (359)
	25% $(CH_3)_2NH$ aq.	--	+0.13 (378), -2.14 (340)	--	--
	DMF	--	+0.68 (385), -0.60 (372)sh, -1.08 (358)	--	--
	DMSO	14/86	+0.73 (383), -0.54 (368)sh, -1.02 (356)	--	128 (362)
	acetone	--	+0.58 (387), -0.87 (372), -1.50 (360)	--	124 (377) 162 (363)
	2-chloroethanol	--	+0.06 (385), -1.41 (343)	--	--

[a] Z/E ratio determined by ^1H NMR for 9 - 12 and by ^{13}C NMR (Ref. 32) for 13. Compounds 9 and 13 are liquids and compounds 10, 11, and 12 were equilibrated before spectra were obtained.

[b] These samples of differing Z,E ratios were obtained by recrystallization from acetone-water (Z/E : 37/63) and chloroform-ether (Z/E : 30/70).

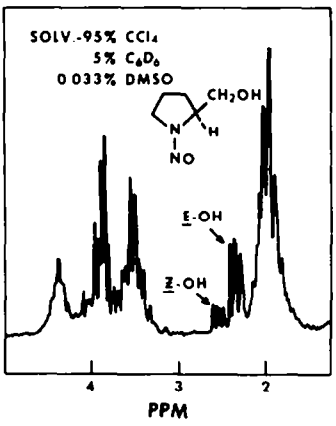


Fig. 3. ^{1}H NMR spectrum of 13 in 95% CCl_4/5% C_6D_6 with 0.033% DMSO added. Solute concentration is 0.030%.

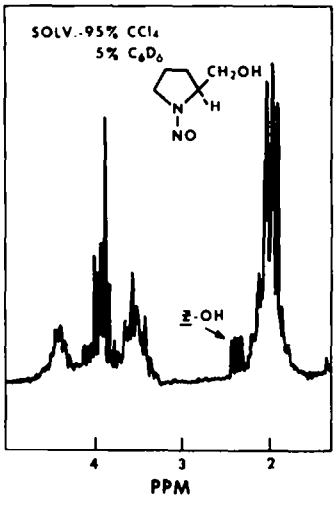
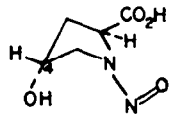
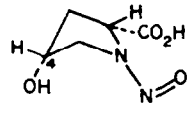


Fig. 4. ^{1}H NMR spectrum of 13 in 95% CCl_4/5% C_6D_6. Solute concentration is 0.016%.

Extensive studies on the geometry of the pyrrolidine ring by a variety of technqiues have shown that in most cases the 5-membered ring is not planar. The pyrrolidine ring in poly-L-proline has been found[14] to exist in solution as two equally populated conformers, one with the C-4 carbon atom slightly above the plane of the remaining four ring atoms and one with C-4 below the ring plane. These conformations rapidly interconvert via the planar conformation so that effective planarity may be achieved on the average. By contrast, the 4-hydroxypyrrolidine ring in poly-*trans*-4-hydroxy-L-proline is found[15] to preferentially populate one ring conformation, with C-4 below the plane of the ring, i.e. exo (*trans*) to the carboxyl group. Similarly, *cis*- and *trans*-4-fluoro-L-proline exist in solution predominantly as a single envelope conformation with C-4 above and below the ring plane, respectively.[16] The contribution of the skewed C atoms of cyclopentanone rings to the CD of ketones has been shown[17] to outweigh second order effects due to asymmetric substituents, and certain lactones have been studied[18] in which the chiroptical properties are dominated by skeletal rather than substituent effects. Thus, it seemed worthwhile to examine the CD of N-nitrosopyrrolidines isomeric at C-4. Careful analysis of the proton NMR spectrum of **2** gave values (Table 3) for coupling constants similar to those reported by Pogliani and Ellenberger[19] for *trans*-4-hydroxy-L-proline (*cf* NMR data[20] for *cis*-4-hydroxy-L-proline). This indicates that nitrosation of the amino nitrogen of *trans*-4-hydroxy-L-proline has little effect upon the conformation of the pyrrolidine ring; assuming that nitrosation of the amino group of *cis*-4-hydroxy-D-proline does not affect the conformation of the *cis*-isomer any more than it does the *trans*, then the ring conformations of *Z*-**2** and *Z*-**5** may be represented as shown below. Observation of CD



Z-**2**



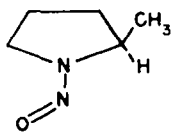
Z-**5**

bands of positive and negative sign for **2** and **5**, respectively, indicates that ring puckering at C-4 is less important than the configuration of the α-carboxyl group in determining the sign of the $n \rightarrow \pi^*$ CD band.

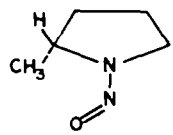
DISCUSSION

The CD properties of a series of N-nitrosopyrrolidines and N-nitrosamino acids have been studied in an attempt to resolve apparent conflicts between the sector rules advanced by Snatzke *et al.*[3] (with the signs proposed by Gaffield *et al.*[5]) on the one hand and Połoński and Prajer[8] on the other. Each rule proposed four sectors, A, B, C and D, located above the plane containing the C_1C_2NNO atoms and four sectors directly below each of these, A', B', C' and D', respectively. The two sector rules are represented graphically in Fig. 5 which shows the differences in nodal surface locations between them.

Exceptions to existing rules. Most of the data reported herein and elsewhere on the $n \rightarrow \pi^*$ CD properties of N-nitrosamines can be rationalized by either sector diagram. However, as reported previously[7] by Ringdahl and Dahlbom and confirmed herein, observation of a positive $n \rightarrow \pi^*$ CD band for N-nitroso-2(R)-methylpyrrolidine (**9**)86% *E*, 14% *Z*) is incompatible with the rule represented in diagrams 2 of Fig. 5. The positive CD band of **9** is consistent only with a positive sign for sectors A' and B, and with placement of the perturbing Me group in sector B, rather than in sector C as proposed[8] by Połoński and Prajer. On the other hand, as indicated by Liberek *et al.*,[9] the formulation of Snatzke *et al.*[3] is incapable of accommodating the results for *Z*- and *E*-N-nitrosoproline regardless of how the sector signs are specified. Thus, we are forced to agree with Ferber and Richardson[10] that the nitrosamine $n \rightarrow \pi^*$ sector rules published to date lack complete generality.



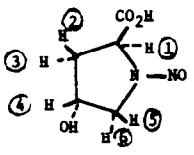
E-**9**(86%)



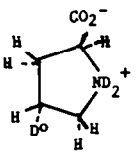
Z-**9**(14%)

The reason for this is not clear, but one possibility could involve distortion of the shapes and locations of nodal surfaces in changing from one structure to another.

Table 3. Comparison of coupling constants[a] of *N*-nitroso-*trans*-4-hydroxy-L-proline with those of *trans*-4-hydroxy-L-proline



NO-L-Hypro



L-Hypro

J	NO-L-Hypro	L-Hypro
1,2	8.94 Hz	10.44 Hz
1,3	8.25	7.66
1,4	0.65	-0.70
1,5	1.43	0
1,6	0.55	0
2,3	-13.25	-14.06
2,4	4.39	4.31
2,5	-0.12	0
2,6	-0.24	0
3,4	2.36	1.41
3,5	-0.10	0
3,6	1.98	1.60
4,5	3.96	4.09
4,6	1.76	1.22
5,6	-12.15	-12.69

a Spectral data on N-nitroso-trans-4-hydroxy-L-proline were obtained at 31° in pyridine-d_5 using a 100 MHz nuclear magnetic resonance spectrometer with internal lock. The data for protons 1-6 were analyzed with the aid of the iterative, least-squares program LAOCOON-3. The parameters listed in this Table produced a PMR spectrum whose peaks had a maximum deviation of 0.1 Hz from those observed experimentally. Data on trans-4-hydroxy-L-proline (L-Hypro) are from L. Pogliani and M. Ellenberger, Spectrosc. Lett. 6, 261 (1973).

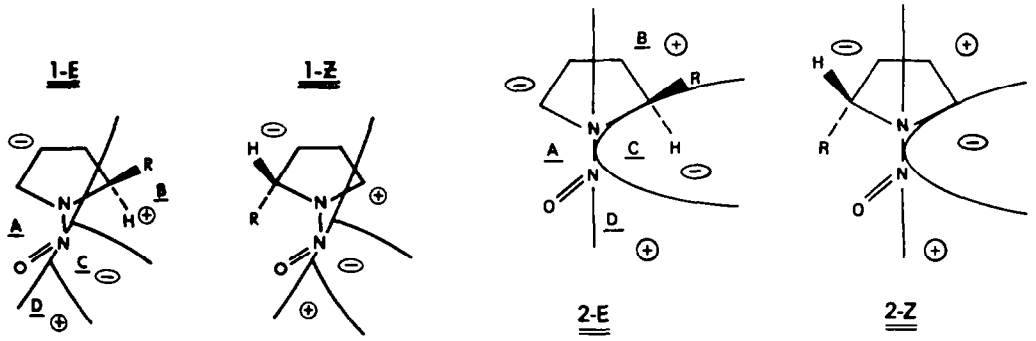


Fig. 5. Sector rules proposed for the $n \rightarrow \pi^*$ transition of the N-nitrosamine chromophore. *A*, *B*, *C* and *D* represent sectors above the plane of the page, and mirror-image sectors are below the page represented by *A'*, *B'*, *C'* and *D'*. 1-*E* and 1-*Z* are for the sector rule of Snatzke *et al.*[3] with the signs proposed by Gaffield *et al.*[5] 2-*E* and 2-*Z* represent the sector rule proposed by Pośniński and Prajer (Ref. [8]).

For example, the nitrosamino group is known to possess a steric requirement large enough within the plane defined by its three atoms (N-N=O) to force bulky substituents alpha to it into the axial conformation.[12,21] Conceivably, the reciprocity of this steric interaction could twist the nitroso group out of the C_1C_2NNO system, whose planarity is assumed in both sector rules shown in Fig. 5. Such an interaction would twist the NNO system in a chiral sense, preventing maximum overlap of its respective molecular orbitals and giving

rise to an inherent dissymmetry of the nitrosamino chromophore which, even if small, could lead to major departures from expectations based upon simple application of either $n \rightarrow \pi^*$ sector rule.

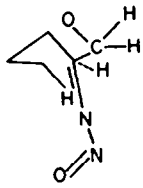
Predictive value of existing rules. Despite the fact that both existing sector rules have proven incompatible with at least some $n \rightarrow \pi^*$ CD data for the nitrosamine chromophore, the basic concept is capable of providing important insights into the stereochemistry of N-nitroso compounds, especially when conclusions are drawn from closely related derivatives. To illustrate the predictive value of the sector rule represented by diagrams 1 of Fig. 5, we cite our conclusion from the CD behavior of **13** in various solvents that the OH group of this compound is capable of intramolecularly H-bonding the nitrosamino N atom, an interpretation which has been supported by subsequent IR and NMR studies.

At the beginning of this investigation, we predicted that all compounds in Table 2 should display $n \rightarrow \pi^*$ Cotton effects of the same sign since their configuration is identical. However, while the CD of **9–12** was generally positive, as predicted, **13** exhibited either negative or bisignate Cotton effects in all eleven solvents studied.

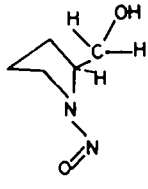
This seemingly anomalous behavior could be explained if one assumed that the OH group of **13** was intramolecularly H-bonded to the amino N in the *E*-conformer, an interaction which would pull the –OH group into a strongly negative contributing portion of sector C (structure 1 – *E* of Fig. 5). In nonpolar solvents, this H-bonded conformation would predominate, giving rise to a negative Cotton effect, as observed. In polar solvents, the constraints of intramolecular H-bonding would be overcome, allowing rotamers having the OH group in the B sector to occur in significant concentrations. Since the various rotamers[22] would be individually active according to the Franck–Condon principle, a net Cotton effect containing both positive and negative components would result if the individual bands were centered at slightly different wavelengths. This behavior is illustrated in Fig. 1, which shows the shift from negative to bisignate Cotton effects on successively adding small amounts of dimethylsulfoxide to CCl_4 solutions of **13**.

To test our interpretation that intramolecular H-bonding was responsible for this behavior, we examined the IR spectra of **13** in CCl_4 at higher dilution, revealing the presence of both free and bonded –OH at 3638 cm^{-1} (66%) and 3550 cm^{-1} (34%), respectively. These spectra were measured at concentrations generally regarded as sufficiently low (~0.065%) to exclude intermolecular association.[23] (Since about 15% of the molecules of **13** are in the *Z*-conformation, the free/bonded ratio of 66/34 indicates that about 40% of the *E*-molecules are intramolecularly bonded.)

Additional evidence for intramolecular H-bonding was obtained from the NMR spectra of **13**. Two distinct OH proton resonances were observed for the *E*- and *Z*-conformers (Figs. 2–4). While the *E*/*Z* ratio of **13** proved to be relatively insensitive to solvent (14–20% of *Z* in each case studied, see Table 2), the OH proton resonance moved much more rapidly downfield for the *E*-conformer upon addition of DMSO to its solutions in nonpolar media (Figs. 2–4) than did the signal for the *Z*-conformer. This observation is also consistent with intramolecular H-bonding in *E*-**13** which should position the –OH proton above the plane of the nitrosamino (*cf* *E*-**13a**) group and thus in a zone[24] of shielding influence.† Upon addition of DMSO, the intramolecular association is inhibited, resulting in a greater percentage of rotamers in which the OH group is remote from the shielding zone, possibly interacting with one or more molecules of solvent[26] (*cf* *E*-**13b**). Inspection of models indicates that H-bonding between the OH group and the amino N should be much less important in the *Z*-conformer, accounting for its relative lack of chemical shift change upon DMSO addition.



E-**13a**



E-**13b**

The only other exception to our initial prediction that the $n \rightarrow \pi^*$ Cotton effect for nitrosopyrrolidines **9–12** should all be positive was the strongly negative CD observed for **12** in chloroform solution. This finding may reflect a propensity of the benzyl group to protrude into Sector C under certain conditions.

A $\pi \rightarrow \pi^*$ rule? Perhaps the most important outcome of the present study was that all seven N-nitrosamino acids having the L-proline configuration at C-2 (**1**, **2**, **3**, **4**, **6**, **7** and **8**) gave negative CD bands at the nitrosamine $\pi \rightarrow \pi^*$ transition[27] near 240 nm, while **5**, of opposite configuration at C-2, gave a positive $\pi \rightarrow \pi^*$ band. In addition, N-nitrosopyrrolidines **9**, **10**, **11**, **12** and **13**, having the L-proline configuration at C-2, also gave negative CD bands at 232–242 nm (Table 2).

We thus tentatively conclude that the $\pi \rightarrow \pi^*$ transition may offer a more reliable method for assigning stereochemistry in optically active N-nitroso compounds than the $n \rightarrow \pi^*$ formulations have provided. We recommend that future investigators collect data on the chiroptical properties of the nitrosamine chromophore's $\pi \rightarrow \pi^*$ transition whenever possible, so that its potential generality can be submitted to a proper test based upon more than the 13 model compounds reported herein.

EXPERIMENTAL

Due to the toxic and carcinogenic properties of N-nitroso compounds, utmost caution must be exercised in their preparation and handling. Appropriate precautions have been described (cf J. Chem. Ed. **52**, A 419 (1975)). B. and m.p. are uncorr; m.ps were obtained with a Thomas–Hoover capillary m.p. apparatus. CD spectra were obtained with the aid of a Cary 6003 dichrometer equipped with a Haake Model KT-62 circulating bath for measurements at 0°. The range of concentrations studied was from 0.014 to 9.4% with most measurements performed using concentrations of 0.08–0.25%. ^{1}H NMR spectra

†Although there are a large number of contributions to the –OH chemical shift accompanying interactions such as hydrogen bonding and therefore it is unlikely that the hydrogen bonding contribution can be separated from the other terms, the chemical shift is shifted downfield as a result of intramolecular hydrogen bonding.[25] The observation (Figs. 3 and 4) of the *E*-OH resonance occurring at a higher field than that of the *Z*-OH is the first instance to our knowledge of the ^{1}H NMR signal of the α-substituent of an N-nitrosamine appearing upfield in the *E*-isomer rather than in the *Z*-isomer.

were measured, with the assistance of Ms. Mabry Benson, on a Varian HA-100 spectrometer using TMS as the internal standard. Coupling constants (J) are first order approximations. The ^{13}C and dilute soln ^1H NMR spectra were measured at 25.03 and 99.5 MHz, respectively, on a JEOL JNM-PFT 100 spectrometer equipped with an EC-100 data system with 32K memory at a probe temp of 29° unless otherwise stated. ^{13}C NMR chemical shifts are reported in δ units using TMS as internal standard. The dilute soln IR spectrum of **13** was recorded on a Cary Model 90 spectrophotometer in carefully dried CCl_4 by Ms. Saima Kint; areas of the free and bonded OH bands were estimated by weighing cut-outs of Xerox copies of the plotted bands. Mass spectra were measured on a JEOL-JMS-01SG-2 Mass Spectrometer at 70 eV by Dr. P. P. Roller (NIH). Elemental microanalyses were determined by Ms. Geraldine Secor.

Reference to a company and/or product by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Preparation of N-*nitrosamines.* An equimolar secondary amine-HCl soln, prepared using the minimum amount of water needed to dissolve the reactant, was cooled to 0–5° in an ice bath. To this soln was added, slowly with stirring, a two-fold excess of $NaNO_2$ dissolved in a few ml of water. After stirring the mixture for 1 hr and allowing it to stand overnight at ambient temp, the N-nitrosamine separated from the soln either as a solid ppt or as a yellow oil. Solid products were removed by filtration and recrystallized from appropriate solvents. Oils were extracted from the aq mixture by CH_2Cl_2; the extracts were dried over Na_2SO_4 and the N-nitrosamine was purified by vac distillation after solvent removal. Liquid N-nitrosamines were dried over Linde Molecular Sieves (4–8 mesh) while solids were vac dried over P_2O_5 prior to analyses.

1-*Nitroso*-2(R)-*methylpyrrolidine* (**9**). 2(*R,S*)-Methylpyrrolidine (Ames Laboratories) was resolved with D-tartaric acid to give a D-tartrate salt, $[\alpha]_D^{27}$ + 17.0(H_2O) (lit.[28] $[\alpha]_D^{21}$ + 17.0). Liberation of the amine followed by distillation gave (−)-2(*R*)-methylpyrrolidine, b.p. 92–94°, $[\alpha]_D^{27}$ − 13.3(H_2O), (lit.[28] b.p. 94°/728 Torr, $[\alpha]_D^{22}$ − 11.97(H_2O)).

Nitrosation of (−)-2(*R*)-methylpyrrolidine afforded **9**, b.p. 104–105°/18 Torr; δ(C_5D_5N) 4.68–4.18 (m, 1.3H, H-2, 0.15 (*Z*-H-5 + *Z*-H-5')), 3.78–3.58 (m, 1.7H, 0.85 (*E*-H-5 + *E*-H-5'), 2.43–1.60 (m, 4H, H-3, H-3', H-4, H-4'), 1.53 (d, 2.58H, ^3J = 6.0 Hz, *E*-CH_3), 1.24 (d, 0.42H, ^3J = 6.0 Hz, *Z*-CH_3); MS: *m/e* 114[*M*$^+$](3), 99(1), 84(5), 83(5), 69(41), 68(25), 42(39), 41(100); Found: C, 52.3; H, 8.88. $C_5H_{10}N_2O$ requires: C, 52.61; H, 8.83%.

1-*Nitroso*-2(S)-*pyrrolidinecarboxylic acid methyl ester* (**10**). Nitrosation of L-proline methyl ester (Sigma) gave **10**, b.p. 99–100°/0.25 Torr, (lit.[4] 160°/20 Torr, lit[8] $[\alpha]_D^{25}$ − 146(dioxane)); δ(C_5D_5N) 5.44 (t, 0.35H, ^3J = 6.0 Hz, *E*-H-2), 4.75–4.54 (m, 0.65H, *Z*-H-2), 4.40–4.19 (m, 1.3H, 0.65 (*Z*-H-5 + *Z*-H-5')), 3.75-3.54 (m, 3.7H, O-CH_3 + 0.35 (*E*-H-5 + *E*-H-5')): 2.36–1.62 (m, 4H, H-3, H-3', H-4, H-4'); MS: *m/e* 128(1), 69(51), 68(28), 44(51), 42(39), 41(100); Found: C, 45.4; H, 6.34. $C_6H_{10}N_2O_3$ requires: C, 45.57; H. 6.37%.

1-Nitroso-2(S)-*pyrrolidine carboxamide* (**11**). Nitrosation of L-proline amide (Sigma) gave **11**, m.p. 165–166°; δ(C_5D_5N) 5.57–5.40 (m, 0.34H, *E*-H-2), 5.02–4.77 (m, 0.66H, *Z*-H-2), 4.44–4.21 (m, 1.3 H, 0.65 (*Z*-H-5 + *Z*-H-5')), 3.80–3.60 (m, 0.70H, 0.35 (*E*-H-5 + *E*-H-5')), 2.46–1.82 (m, 4H, H-3, H-3', H-4, H-4'); MS: *m/e* 143 [M$^+$](15), 113(5), 99(79), 70(100), 69(70), 44(54); Found: C, 42.4; H, 6.48. $C_5H_9N_3O_2$ requires: C, 41.95; H, 6.34%.

1-*Nitroso*-L-*proline benzyl ester* (**12**). Nitrosation of L-proline benzyl ester (Sigma) gave **12**, m.p. 93.0–93.5°; δ(C_5D_5N) 7.33 (s, 5H, C_6H_5–), 5.51 (t, 0.70H, ^3J = 5.0 Hz, *E*-H-2), 5.23 (s, 2H, C_6H_5–CH_2–), 4.84–4.64 (m, 0.30H, *Z*-H-2), 4.35–4.18 (m, 0.60H, *Z*-H-5, *Z*-H-5'), 3.74–3.39 (m, 1.40H, *E*-H-5, *E*-H-5'), 2.34–1.59 (m, 4H, H-3, H-3', H-4, H-4'); MS: *m/e* 234[M$^+$](5), 204(1), 99(67), 91(100), 69(17); Found: C, 61.7; H, 6.03. $C_{12}H_{14}N_2O_3$ requires: C, 61.53; H, 6.02%.

1-*Nitroso*-cis-4-*hydroxy*-D-*proline* (**5**). Nitrosation of *cis*-4-hydroxy-D-proline (Sigma) gave, after recrystallization from acetone, **5**, m.p. 126–127°; δ(C_5D_5N) 5.74 (dd, 0.25H, ^3J = 11.0 Hz, ^3J = 2.5 Hz, *E*-H-2), 5.20–4.00 (m, 3.75H, *Z*-H-2, H-4, H-5, H-5'), 3.04–2.34 (m, 2H, H-3, H-3'); MS: *m/e* 160[M$^+$](9), 130(12), 115(62), 80(72), 68(48), 56(100), 51(40), 44(96); Found: C, 37.6; H, 5.17. $C_5H_8N_2O_4$ requires: C, 37.50; H, 5.04%.

1-*Nitroso*-trans-4-*fluoro*-L-*proline* (**6**). Nitrosation of *trans*-4-fluoro-L-proline,[16] a gift from Prof. J. T. Gerig, gave **6**, m.p. 143–145°; MS: *m/e* 162[M$^+$](62), 145(3), 132(2), 117(100), 88(20), 87(22), 68(20), 59(30), 44(34).

1-*Nitroso*-cis-4-*fluoro*-L-*proline* (**7**). Nitrosation of *cis*-4-fluoro-L-proline,[16] a gift from Prof. J. T. Gerig, gave **7**, m.p. 127–128°; MS: *m/e* 162[M$^+$](42), 117(82), 88(21), 68(18), 59(20), 45(22), 44(21).

1-*Nitroso*-2(S)-*pyrrolidinemethanol* (**13**). Nitrosation of 2(*S*)-pyrrolidinemethanol(Aldrich), $[\alpha]_D^{27}$ + 3.6° (MeOH, *c* = 4.8), $[\alpha]_D^{27}$ + 4.54° (neat), $[\alpha]_D^{27}$ + 1.5° (EtOH, *c* = 3.6), $[\alpha]_D^{27}$ + 10.0° (H_2O, *c* = 3.0); (*lit.* $[\alpha]_D^{20}$ + 3.38° (MeOH)[29], $[\alpha]_D^{19.5}$ + 2.0° (EtOH)[30], $[\alpha]_D$ + 10° (H_2O)[31]), afforded **13**, b.p. 119–120°/0.5 Torr, (lit.[8] oil, $[\alpha]_D^{25}$ − 138° (EtOH)); IR (CCl_4, 3.8×10^{-3} *M*, 0.05%) ν_{max} 3638, 3550 cm^{-1} (similar results were obtained for concn = 0.1, 0.2 and 0.4%); ^1H NMR δ(C_5D_5N) 4.79–4.48 (m, 0.93H, *E*-H-2), 4.40–3.40 (m, 4.1H, *Z*-H-2, H-5, H-5', –CH_2OH), 2.32–1.47 (m, 4H, H-3, H-3', H-4, H-4'); δ(CCl_4) 4.58–4.30 (m, 1H, *E*-H-2), 4.07 (dd, 1H, ^2J = 11.0 Hz, ^3J = 3.5 Hz, –CH_2OH), 3.82 (dd, 1H, ^2J = 11.0 Hz, ^3J = 3.5 Hz, –CH_2OH), 3.70–3.40 (m, 2H, H-5, H-5'), 3.39 (s, 1H, –OH), 2.35–1.75 (m, 4H, H-3, H-3', H-4, H-4'); δ($CDCl_3$) 4.70–4.29 (m, 1H, *E*-H-2), 4.20–3.31 (m, 5H, H − 5, H-5', –OH, –CH_2OH), 2.41–1.67 (m, 4H, H-3, H-3', H-4, H-4'); δ(DMSO-d_6) 5.02 (t, 0.83H, ^3J = 5.5 Hz, *E*-OH), 4.80 (t, 0.174, ^3J = 5.5 Hz, *Z*-OH), 4.66–4.33 (m, 0.86H, *E*-H-2), 4.30–3.23 (m, 4.1H, *Z*-H-2, H-5, H-5', –CH_2OH), 2.25–1.67 (m, 4H, H-3, H-3', H-4, H-4'); ^1H NMR (dil soln, Figs. 2–4) δ(95% CCl_4 − 5% C_6D_6 containing 0.033% DMSO-d_6, solute concn = 0.030%) 2.51 (dd, 0.24H, ^3J = 4.5 Hz, 7.2 Hz, *Z*-OH), 2.29 (dd, 0.76H, ^3J = 5.3 Hz, 7.6 Hz, *E*-OH); δ(95% CCl_4 − 5% C_6D_6, solute concn = 0.016%) 2.29 (dd, ^3J = 4.3 Hz, 7.1 Hz, *Z*-OH); δ(75% DMSO-d_6-25% CCl_4, solute concn = 0.050%) 4.92 (t, ^3J = 6.0 Hz, *E*-OH), 4.70 (t, ^3J = 6.0 Hz, *Z*-OH); ^{13}C NMR δ(95% CCl_4 − 5% C_6D_6) 63.6 (0.82 C, *E*-α-CH_2-OH), 61.6 (0.18 C, *Z*-α-CH_2-OH), 62.4 (0.85 C, *E*-C-2), 59.6 (0.15 C, *Z*-C-2), 50.5 (0.17 C, *Z*-C-5), 45.7 (0.83 C, *E*-C-5), 26.5 (C-3), 22.6 (0.16 C, *Z*-C-4), 21.0 (0.84 C, *E*-C-4), avg. % *Z*-isomer = 16.8 ± 1.3, avg. dev., at 65° avg. % *Z*-isomer = 21.0 ± 0.7, avg. dev.; δ(DMSO-d_6) 62.7 (0.86 C, *E*-α-CH_2OH), 59.0 (0.14 C, *Z*-α-CH_2OH), 62.0 (0.86 C, *E*-C-2), 58.5 (0.14 C, *Z*-C-2), 50.3 (0.14 C, *Z*-C-5), 45.7 (0.86 C, *E*-C-5), 26.3 (0.81 C, *E*-C-3), 25.8 (0.19 C, *Z*-C-3), 22.0 (0.15 C, *Z*-C-4), 20.3 (0.85 C, *E*-C-4); δ(C_6D_6) 64.3 (*E*-α-CH_2OH), 62.9 (*Z*-α-CH_2OH), 62.6 (*E*-C-2), 60.3 (*Z*-C-2), 50.7 (0.17 C, *z*-C-5), 45.9 (0.83 C, *E*-C-5), 26.5 (C-3), 22.4 (0.21 C, *Z*-C-4), 20.9 (0.79 C, *E*-C-4); δ($CDCl_3$) 64.2 (*E*-α-CH_2OH), 63.3 (*Z*-α-CH_2OH), 63.J (*E*-C-2), 61.0 (*Z*-C-2), 51.4 (0.19 C, *Z*-C-5), 46.3 (0.81 C, *E*-C-5), 26.9 (*Z*-C-3), 26.7 (*E*-C-3), 22.7 (0.18 C, iZ-C-4), 21.1 (0.82 C, *E*-C-4); MS: *m/e* 130 [M$^+$](22), 113(15), 100(11), 99(100), 70(56), 69(79), 68(29), 55(23), 42(27), 41.43); high resolution MS: 113.0695 ($C_5H_9N_2O$, M$^+$–OH), 101.0715 ($C_4H_9N_2O$, M$^+$–CHO), 100.1655 ($C_4H_8N_2O$, M$^+$–CH_2O), 99.0544 ($C_4H_7N_2O$, M$^+$–CH_2OH), 98.0606 (C_5H_8NO, M$^+$–H_2NO); Found: mol. wt. 130.0747, $C_5H_{10}N_2O_2$ requires: 130.0742.

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