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THE PREPARATION AND PROPERTIES OF SOME NITROSAMINO ACIDS*

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Abstract—The N-nitroso derivatives of the amino acids sarcosine, azetidine-2-carboxylic acid, proline, hydroxyproline and pipecolic acid have been prepared and characterized. All five compounds are formed in high yield under conditions approximating those in the mammalian stomach. The nitrosamino acids can be decarboxylated in low yield in the presence of dilute alkali, the product in each case being a nitrosamine. The possible relevance of these findings to the occurrence of human cancer is discussed. The variation of NMR spectral properties with time has shown that four of these nitrosamino acids preferentially crystallize in a conformation in which the C atom bearing the carboxyl group is *syn* to the nitroso O atom, while nitrosoazetidinecarboxylic acid assumes the *anti* conformation in the crystal. The carboxyl group of nitrosopipecolic acid strongly prefers the axial orientation.

ALTHOUGH the existence in nature of N-nitroso derivatives of those amino acids which are secondary amines has not been reported, these compounds might be of significance as environmental carcinogens because of the possibility that they can be formed from the amino acids and nitrite in the mammalian stomach.[1] The possible relevance of these nitrosamino acids to human cancer has led us to prepare some of them and to begin testing them for carcinogenic activity by long-term administration to animals.

There appeared to be significant differences in properties between the compounds prepared by us and those reported in the literature. There have been several references to the preparation of nitrosoproline (II),[2–4] nitrosohydroxyproline (III)[3,4] and nitrosopipecolic acid (IV),[5,6] but there are substantial variations in the reported properties of the compounds. We have attempted to reconcile these differences by reinvestigating the preparation and properties of nitrosoproline, nitrosohydroxyproline and nitrosopipecolic acid, and have included two additional nitrosamino acids of possible significance as environmental carcinogens, nitrososarcosine (I) and nitrosoazetidine-2-carboxylic acid (V). The latter is of interest because azetidinecarboxylic acid is a naturally occurring antimetabolite of proline, which might be used for the treatment of schistosomiasis in man. Some sarcosine derivatives are used in toothpaste.

The previously described preparations of nitrosoproline and nitrosohydroxyproline give little assurance of the purity of the products;[2–4] nitrosopipecolic acid was described as an oil,[5] and as a solid melting at 99°.[6] There is considerable disparity in

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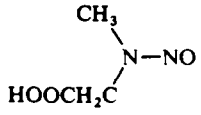
m.p. between the nitrosoproline and nitrosohydroxyproline reported by Heyns and Königsdorf[3] (120° and 126°, respectively) and the corresponding compounds prepared by us; the properties of the former two compounds are also at variance with ours, especially in that our compounds are quite stable at room temp and our nitrosohydroxyproline is almost insoluble in ether and cannot be extracted from aqueous medium with this solvent. The nitrosoproline prepared by Sander[2] appeared to be similar to that from our preparation except that the former was brown, possibly a



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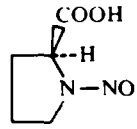

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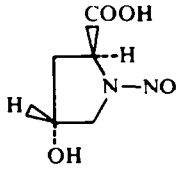
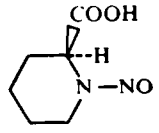
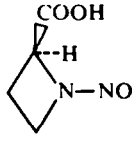


III



IV



V

consequence of crystallization from benzene, while our compound was colorless; crystallization of our nitrosoproline from benzene also gave a brown solid, smelling of nitrogen dioxide. Nitrososarcosine has been described both as an oil and as a solid.[6]

RESULTS AND DISCUSSION

The amino acids were converted easily into the N-nitroso derivatives by reaction with sodium nitrite in dilute acid solution; the yields were high. It can be inferred that such a reaction could take place in the mammalian stomach, more particularly in the stomach of man, whose diet can contain nitrites and free amino acids, which are found in blood and muscle.

Nitrosoproline, nitrosohydroxyproline, nitrosopipecolic acid, nitrosoazetidine-carboxylic acid and nitrososarcosine are colorless crystalline solids which melt sharply with decomposition. Nitrosoproline, nitrosohydroxyproline and nitroso-pipecolic acid suffered decomposition, with discoloration, on heating for some time at 100°, but were quite stable at room temperature. All five nitrosamino acids were highly soluble in water and most organic solvents, excepting nitrosohydroxyproline, which was almost insoluble in chloroform or ether. Crystallization of the acids from hot alcohols led to almost complete conversion to the corresponding esters, which

TABLE 1. PARTITION OF NITROSAMINO ACIDS

	Conc. in water / Conc. in CH_2Cl_2	Conc. in water / Conc. in ether
Nitrosoazetidine-carboxylic Acid	47	16·5
Nitrosoproline	31	9·2
Nitrosohydroxyproline	--	30
Nitrosopipecolic Acid	3·8	0·8
Nitrososarcosine	100	7·6

TABLE 2. ABSORPTIVITY OF NITROSAMINO ACIDS
$\lambda_{max}(\varepsilon)$

Solvent	Nitrosoazetidine-carboxylic acid	Nitrosoproline	Nitrosohydroxy-proline	Nitrosopipecolic acid	Nitrososar-cosine
Water	343(94)	343(91)	345(92)	345(91)	340(86)
	240(6700)	238(6500)	236(6900)	240(7300)	233(6200)
Ethanol	358(87)	354(100)	354(93)	355(93)	352(93)
	241(6200)	235(6700)	236(6400)	240(6900)	233(6300)
Methylene Chloride	362(108)	361(113)		361(112)	358(105)
	243(6100)	237(6600)		238(6900)	233(5800)
Ether	383(93)	383(87)		373(104)	373(91)
	372(99)	368(109)		362(109)	361(108)
	358(72)	358(87)		350(79)	352(85)
	241(6200)	235(6600)		238(6900)	234(5400)

were non-crystallizing oils and were identified by mass spectrometry. Partition coefficients for the nitrosamino acids are given in Table 1. Nitrosoazetidinecarboxylic acid, nitrosoproline, nitrosohydroxyproline and nitrosopipecolic acid were optically active, with specific rotations more negative than those of the corresponding amino acids; optically active nitrosopipecolic acid was prepared from the racemate by crystallization of the quinine salt. Crystallization of the quinine salt seemed to be a general way of isolating the L-isomer of acids in this series, the salt of the L-acid being much less soluble than the quinine salt of the D-acid.

The nitrosamino acids are strong organic acids, with p*Ka*'s ranging from 3·2 for I to 2·8 for V. They show all of the spectral properties of nitrosamines, having two absorption bands in water, one near 345 mμ ($\varepsilon \simeq 100$) and the other near 238 mμ ($\varepsilon \simeq 10{,}000$). The spectra are almost unchanged in alkali (pH = 11). The absorption shows a bathochromic shift in ethanol or methylene chloride (Table 2), while in ether or benzene the longer wavelength band is shifted to still higher wavelengths and becomes a triplet. The IR spectra of the nitrosamino acids are quite similar, with absorption near 1450 cm^{-1} and 1730 cm^{-1} (due to N=O and C=O, respectively) as prominent features.

The mass spectra of the nitrosamino acids are quite straightforward. The spectra of samples of the same acid, but of different m.p., showed that the same pure compound was present in every case, as was indicated also by the elemental analyses. There was, typically, a strong parent ion in the mass spectrum. The nitroso acids, with the exception of nitrosoproline, showed a p-30 ion, corresponding to loss of —NO. All showed a p-45 ion, corresponding with loss of —COOH (fragmentation of nitrosopipecolic acid-2-d confirmed these findings).

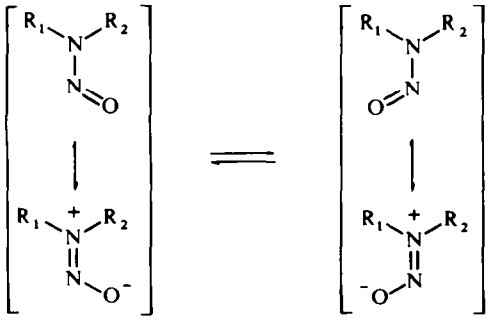
One property of the nitrosamino acids with possible toxicologic consequences is their decarboxylation in dilute alkali (pH 9) to form N-nitrosamines, which are known to be both acutely toxic and carcinogenic. Such a reaction could take place in the alkaline regions of the alimentary tract if a nitrosamino acid were present, or perhaps elsewhere in the presence of an appropriate decarboxylase, and this possibility is being studied experimentally. In no case has it been possible to obtain quantitative decarboxylation of a nitrosamino acid, and the reasons for this are being sought. The decarboxylation was first noticed by the smell of nitrosopyrrolidine in a slightly alkaline solution of nitrosoproline which had been gently warmed. The nitrosopyrrolidine was identified by high resolution mass spectrometric examination of the parent compound and its fragments; no nitrosopyrrolidine was detected in this way in a strongly alkaline solution of nitrosoproline.

We have observed the denitrosation of nitrosopipecolic acid in chloroform solution on standing at room temperature in the dark, with formation of pipecolic acid. Proline was apparently not formed from nitrosoproline under these conditions; nitrosohydroxyproline and nitrosoazetidinecarboxylic acid are almost insoluble in chloroform. To our knowledge denitrosation by chloroform is not a usual reaction of nitrosamines.

A sample was isolated from a concentrated aqueous solution of nitroso-L-proline after standing in the cold, and was the optically inactive racemate.

CONFORMATIONAL STUDIES

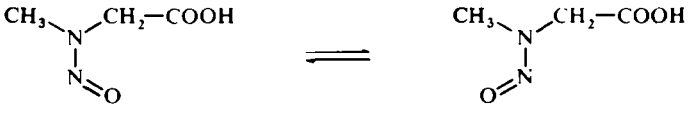
Because of the partial double bond character of its formally single N—N linkage, the nitrosamino group assumes an essentially planar conformation, in which the O atom is *syn* to one substituent and *anti* to the other. The substituents are in general



magnetically non-equivalent and when $R_1 \neq R_2$, two isomeric conformations are possible, which are generally distinguishable by means of NMR spectrometry.[7]

In the nitrosamino acid series, the interconversion of the two conformers, which

results from a rotation of 180° about the N—N bond, is ordinarily sufficiently slow that the return to equilibrium, following some perturbation, can be studied conveniently at room temperature by repetitively scanning the NMR spectrum. This fact has permitted us to infer the conformational preferences of most of the crystalline compounds from solution data alone,* by determining the *syn/anti* ratio as a function of time and extrapolating to the moment of dissolution.



Ia Ib

Nitrososarcosine tends to crystallize in the *syn* conformation, Ia. The NMR spectrum of a freshly prepared solution in pyridine-d_5 contained only two major† peaks, a methyl resonance at 6·06 τ, and a methylene absorption at 5·34 τ. On standing, however, new singlets appeared at 6·71 and 4·71 τ, which were attributed to the methyl and methylene substituents, respectively, of the second conformer.

That the original conformer had the *syn* structure, Ia, was established on the basis of the chemical shift arguments of Karabatsos and Taller,[7] who showed that aliphatic protons resonate at higher field when *syn* to the nitroso O atom than when *anti*, except when they lie in or very near the plane defined by the N—N=O group. Thus, the Me substituent of Ia should appear at lower field than that of Ib, while the methylene protons should absorb at higher field in Ia than in Ib.

It might be argued that the considerations outlined by Karabatsos and Taller are not applicable to nitrososarcosine, since these authors did not include any functionalized nitrosamines in their survey. We feel that our extension of their generalizations to the nitrosamino acids is valid, however, for two reasons. In the first place, the differences in chemical shift between the two Me resonances (0·72 ppm§), and also between the methylene peaks (0·67 ppm§), correspond closely to the $\Delta\delta$ values¶ reported by Karabatsos and Taller for purely aliphatic Me and methylene substituents in non-aromatic solvents.

The second reason for our confidence in the spectral assignments described above is that they permit the direction of the shift in equilibrium that is brought about by

* The first successful attempt to elucidate the conformation of a compound in the crystalline phase using this type of NMR approach appears to be that of F. R. Jensen and C. H. Bushweller (*J. Am. Chem. Soc.* **88**, 4279 (1966))

† Small peaks due to about 3% of the *anti* conformer could be seen in the initial spectrum of the nitrososarcosine sample used in this study, although it had been recrystallized to a constant melting point of 66–67°. Previous authors‡ have reported significantly higher melting points for this compound; it is not clear why our sample should differ in this way.

‡ D. L. Hammick and D. J. Voaden[6] found a melting point of 73–74° for nitrososarcosine, while F. Bergel, S. S. Brown, C. L. Leese, C. M. Timmis, and R. Wade, *J. Chem. Soc.* 846 (1963), reported a sample melting at 75–77°.

§ These values were determined in deuterium oxide. The corresponding data for pyridine-d_5 solutions were $\Delta\delta_{-CH_3} = 0{\cdot}65$ and $\Delta\delta_{-CH_2-} = 0{\cdot}63$ ppm.

¶ As defined by Karabatsos and Taller. $\Delta\delta$ is the change in chemical shift experienced by a given proton upon conformational interconversion, i.e. $\Delta\delta_i = \delta_{i\,syn} - \delta_{i\,anti}$ where chemical shifts are expressed in τ units.

TABLE 3, EQUILIBRIUM CONSTANT VALUES (AT 32°) FOR THE INTERCONVERSION OF THE NITROSAMINO ACIDS IN SOLUTION

Reaction	Solvent: pyridine[a]	Solvent: water[b]
Ia ⇌ Ib	---	1·0[c]
Ia(Na Salt) ⇌ Ib(Na Salt)	--	1·2
IIa ⇌ IIb	0·9	0·5
IIIa ⇌ IIIb	0·5	0·4
IVa ⇌ IVb	0·7	0·6
Va ⇌ Vb	1·3	-

[a] 0·5M in 95:5 (w:w) pyridine-d_5:tetramethylsilane.
[b] 0·5M in 95:5 (w:w) deuterium oxide:t-butyl alcohol.
[c] Solution was also 0·5M in sodium chloride.

neutralization of the acid to be predicted correctly. Ionization of the carboxyl group not only increases its degree of solvation,[8] and hence presumably its effective steric size, but also introduces the possibility that electrostatic repulsive interactions might influence the position of equilibrium. In addition, it is conceivable that intramolecular H-bonding might stabilize the acid molecule. All three factors suggest that neutralization ought to increase the relative abundance of *anti* conformer, since intramolecular H-bonding is not possible in Ib, and since the bulky groups, which are also the centers of negative charge, are separated further in the anion of Ib than in that of Ia. The fact that the equilibrium constant (Table 3) for the *syn* ⇌ *anti* interconversion in deuterium oxide solution is significantly greater for the sodium salt of I ($K = 1·2$) than for I itself ($K = 1·0$)* thus confirms our analysis of the nitrososarcosine spectrum.

Nitrosoproline also crystallizes preferentially in the *syn* conformation, IIa. Fig 1 shows that only one conformer could be detected in the NMR spectrum of a sample run within 4 min of dissolution in pyridine-d_5, but that the other conformational isomer was detectable after 10 min at room temperature; the equilibrium mixture (Fig 1c) contained roughly equal amounts of the two conformers.

Our assignments for nitrosoproline are based upon the following considerations. Inspection of Dreiding models suggests that considerable torsional strain would result if the dihedral angle defined by the C—H and N—N bonds were decreased substantially from its relatively strain-free value† of 60°, and none of the three protons alpha to the nitrosamino function would be expected to spend enough time in or near the N—N—O plane to be deshielded by a *syn* nitroso group.‡ Since the methylene

* Enough sodium chloride was added to the solution of the unionized acid to bring it to an ionic strength equivalent to that of the solution of the sodium salt of I

† That the α-methylene protons of II (and also of III and V) are virtually equivalent magnetically (Figs 1, 2, and 5) suggests that these diastereotopically related protons are quite similarly situated, on the average, with respect to the anisotropic influence of the nitrosamino group, i.e., that the average (H—C—N—N) dihedral angle is in fact close to 60°.

‡ Note that this is in apparent contradiction to one of the generalizations of Karabatsos and Taller,[7] who concluded that "when *cis*... to the nitroso oxygen, α-methine protons resonate at lower fields." This seeming anomaly is easily rationalized in terms of the inherent differences between cyclic and acyclic molecules with respect to their ability to assume the pertinent conformations, however. Thus, the conformation in which one α-methine bond lies in the N—N—O plane is probably actually preferred in diisopropylnitrosamine, but sterically impossible in nitrosoproline.

group of the conformer originally present in the crystal appears at roughly 60 Hz. downfield from the methylene group of the conformer produced during the approach to equilibrium, the former must be *anti* to the nitroso oxygen, as in IIa. The appearance of a new methine resonance *ca* 60 Hz downfield from the methine pattern of the

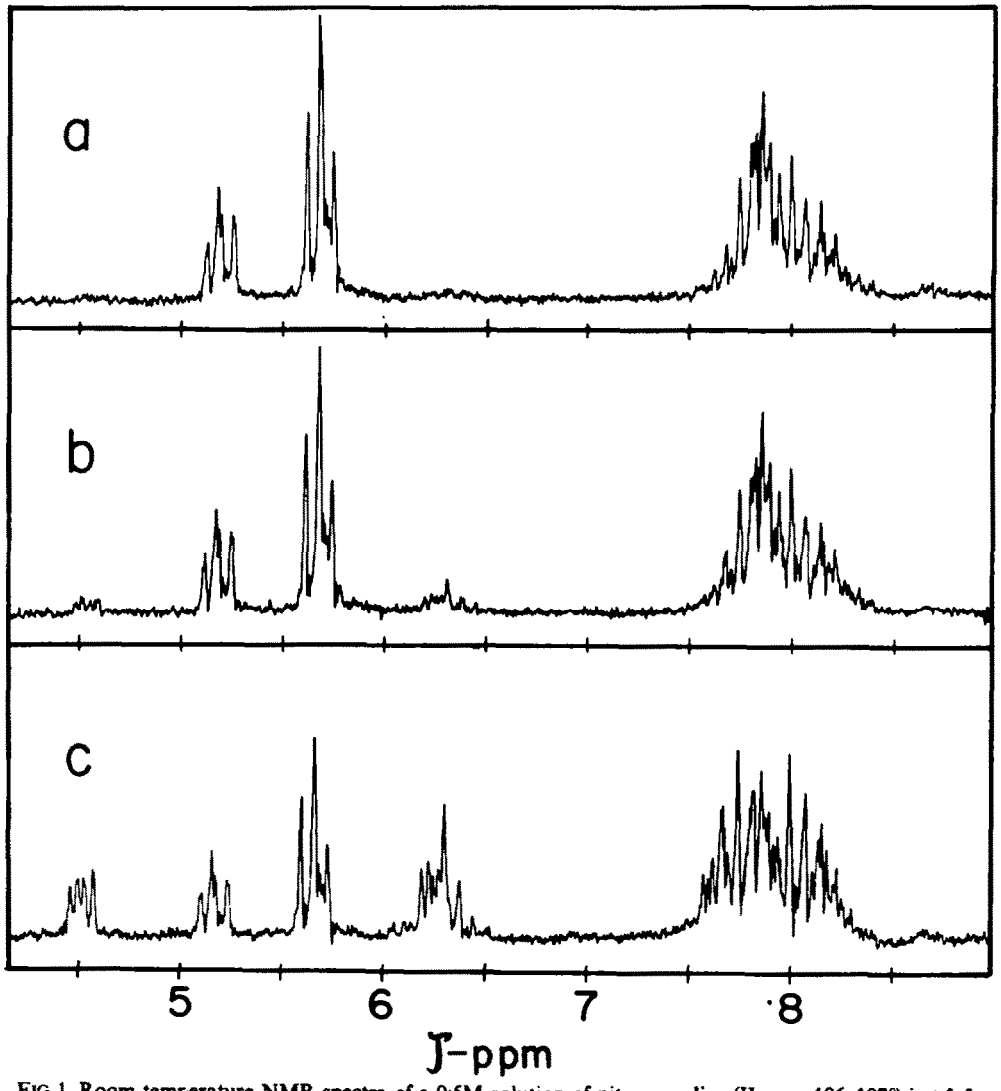


FIG 1. Room temperature NMR spectra of a 0·5M solution of nitrosoproline (II, m.p. 106–107°) in 95:5 (w:w) pyridine-d_5:tetramethylsilane: (a) 4 min after dissolution, (b) 10 min after dissolution, (c) after standing overnight

original conformer similarly supports the conclusion that nitrosoproline crystallizes preferentially in the *syn* conformation, IIa.

Although Karabatsos and Taller did not base their generalizations on data determined using a basic, aromatic solvent such as pyridine, we have confirmed our observations using chloroform-d solutions, in which the methylene resonance moves 70–80 Hz upfield and the methine peak shifts 70–80 Hz downfield upon conformational interconversion.

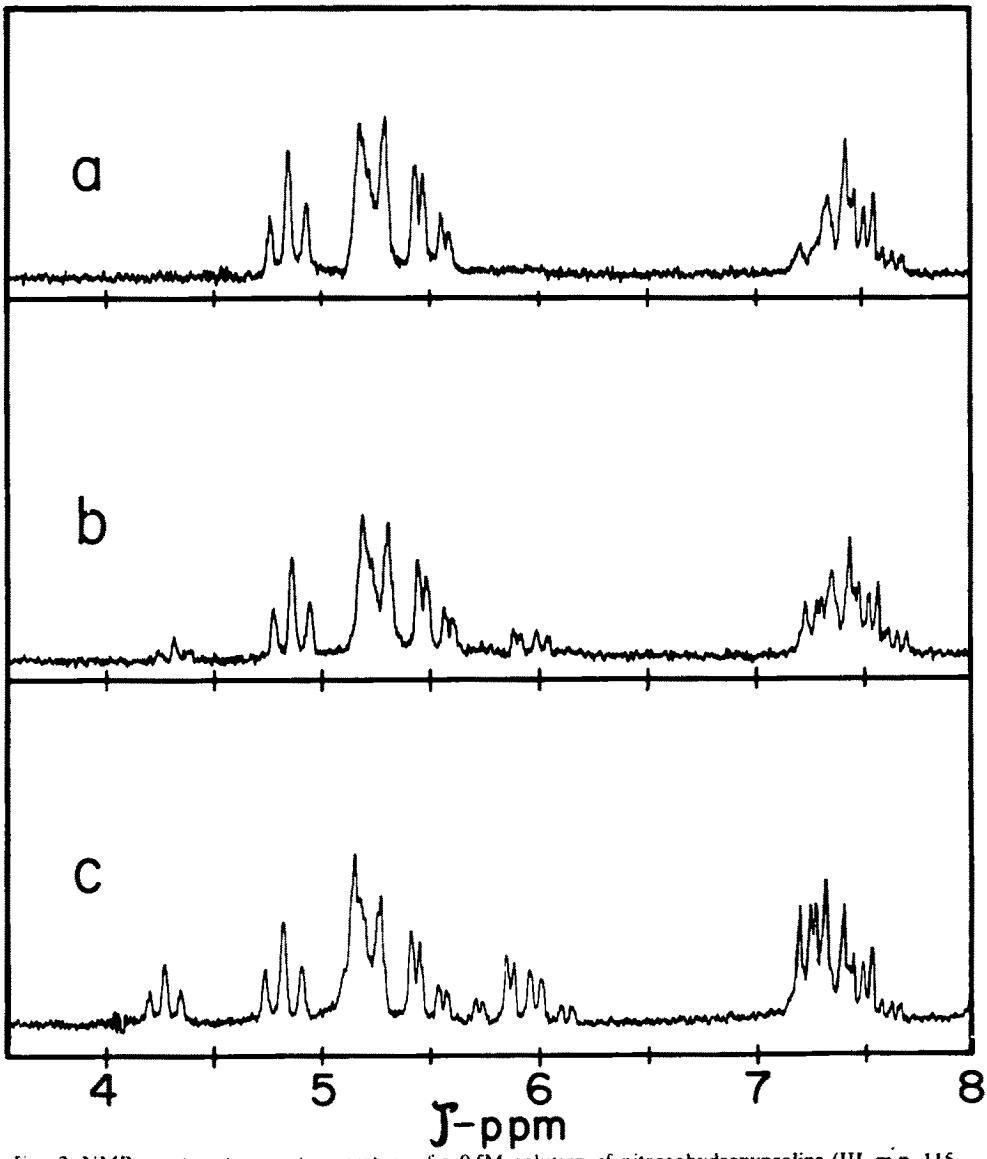


FIG. 2. NMR spectra at room temperature of a 0·5M solution of nitrosohydroxyproline (III, m.p. 115–115·5°) in 95:5 (w:w) pyridine-d_5:tetramethylsilane: (a) 4 min after dissolution, (b) 22 min after dissolution, (c) after standing overnight.

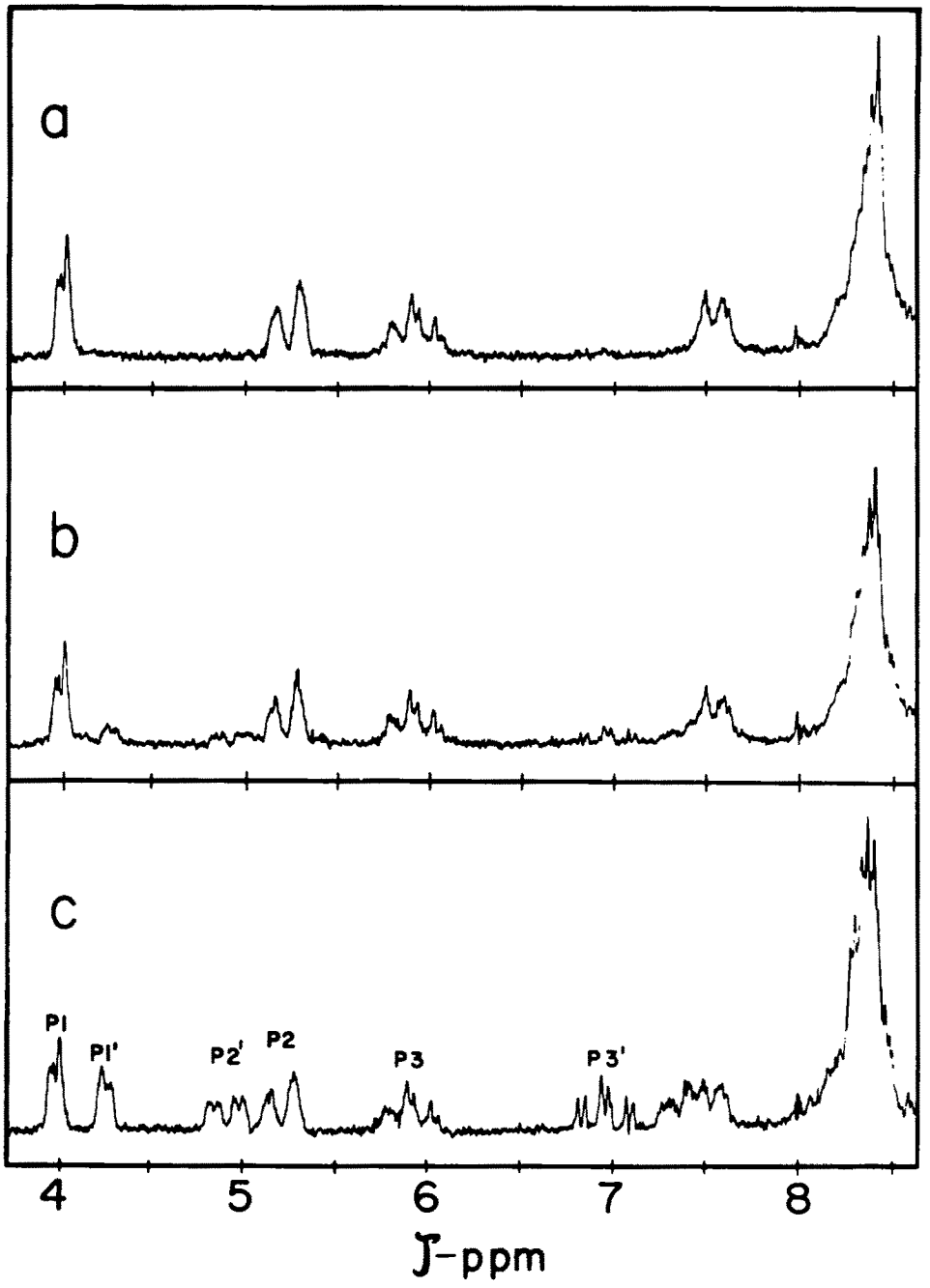
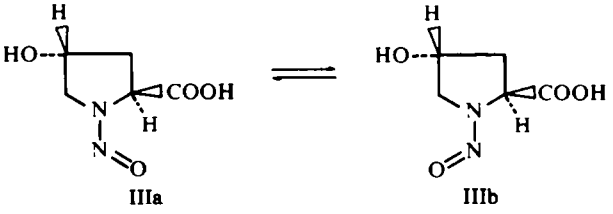


FIG 3. Room temperature NMR spectra of a 0·5M solution of nitrosopipecolic acid (IV, m.p. 89–90°) in 95:5 (w:w) pyridine-d_5:tetramethylsilane: (a) 3 min after dissolution, (b) 38 min after dissolution, (c) after standing overnight.

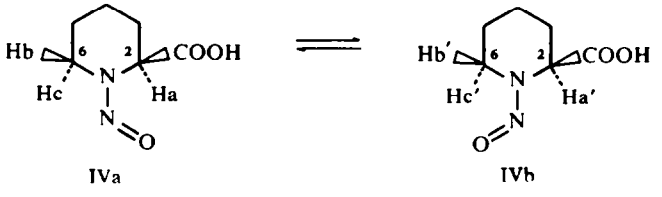
Nitrosohydroxyproline exhibits similar behavior. The NMR spectra (Fig 2) show the gradual appearance in the originally conformationally pure solution of a second conformer, the methine proton of which absorbs at lower field by 55 Hz than that of the original conformer, and the methylene group of which appears upfield from that of the original species by roughly the same amount. Assuming that all of the conformational arguments used for nitrosoproline also apply to its 4-hydroxy derivative, we conclude that nitrosohydroxyproline crystallizes preferentially in the *syn* conformation, IIIa.



IIIa IIIb

Nitrosopipecolic acid (IV) is also conformationally pure in the crystal, as can be seen in Fig 3. The fact that each of the three α-nitrosamino protons gives rise to an isolated pattern complicates the interpretation of these spectra somewhat, but several different, convergent lines of evidence have nevertheless permitted stereochemical assignments to be made unambiguously for this compound.

Of the six different α-nitrosamino resonance patterns found between 4 and 7τ in Fig 3c, two must be due to the 2-methinyl proton. Because of the deshielding influence of the α-carboxyl substituent and the absence of geminal coupling effects, this proton might be expected to appear at lowest field and to give the narrowest pattern in both IVa and IVb. Indeed, both P1 and P1′ (Fig 3c) have widths at half-height of only 8 Hz, but we sought to confirm this assignment by exposing the compound to strongly basic deuterium oxide. Both the carboxyl[9] and nitrosamino* substituents are known to facilitate carbanion formation at the *alpha* position, and we expected the 2-proton to be particularly easily exchanged under these conditions. The isolation of a monodeuterated derivative with an NMR spectrum in which P1 and P1′ are absent (Fig 4b) can therefore be taken as compelling evidence that these peaks are due to the α-methinyl proton.



IVa IVb

Rather surprisingly, the α-methinyl proton appears to be predominantly equatorial in both IVa and IVb. With two vicinal H atoms, this proton should appear as the X part of an ABX system.† As such, theoretical considerations demand that the two strongest lines in the pattern be separated by $|J_{AX} + J_{BX}|$. If the reasonable assump-

* L. K. Keefer, J. Am. Chem. Soc.-in press.

† We have ignored possible contributions of long-range coupling and of virtual coupling effects to the line separations in question. Preliminary attempts at computer simulation of this envelope, for which the authors are indebted to Dr. C. A. Kingsbury, suggest that this approximation is justified.

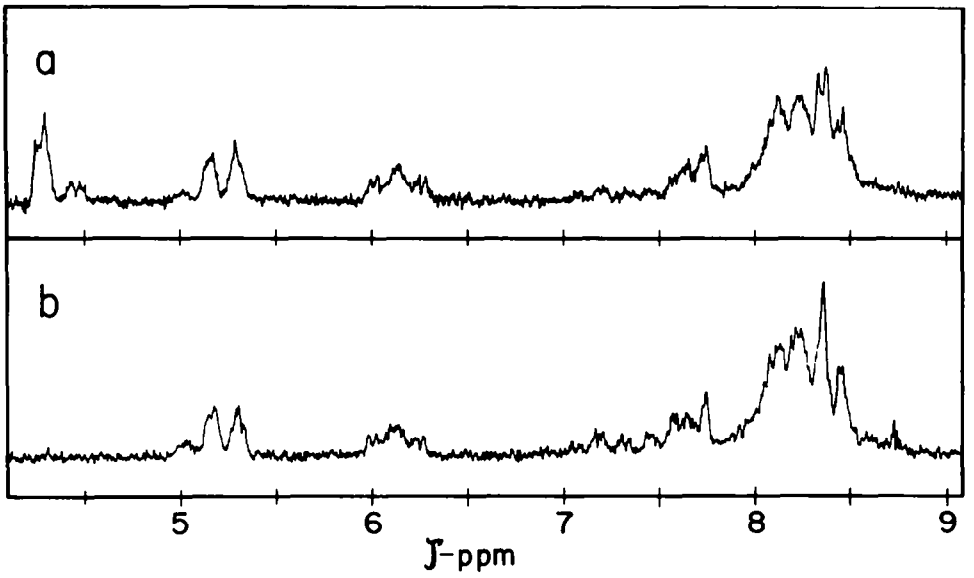


FIG 4. NMR spectra in chloroform-d solution with TMS as internal reference, of: (a) nitrosopipecolic acid (IV, m.p. 89–90°), (b) nitrosopipecolic acid-2-d.

tion is invoked that J_{AX} and J_{BX} are of like sign,[10] then the observed line separations of $\leqslant 5$ Hz for the methine protons of both IVa and IVb (Figs 3 and 4) strongly suggest that both J_{AX} and J_{BX} are small, or, at any rate, that neither is large enough to reflect the strong coupling that would be expected from an axial-axial interaction. Thus, in both conformers, the methine proton must be equatorial, with the carboxyl group axial. This circumstance will be discussed more fully below.

We have assigned P2 and P2′ to the 6-equatorial proton (Hc $\rightleftharpoons$ Hc′) since the appearance of this peak as an approximate doublet suggests that it is not strongly coupled to the vicinal H atoms, i.e., that neither of the corresponding dihedral angles is 180°. The fact that this absorption is downfield from the two remaining, strongly coupled patterns, P3 and P3′, provides additional evidence for the equatorial assignment, since the axial protons might be expected to appear upfield from the corresponding equatorial atoms.

Having assigned the α-nitrosamino proton resonances in both conformers, the conformation of the crystalline compound can now be established on the basis of the chemical shift arguments of Karabatsos and Taller.[7] Like the α-protons of the nitrosopyrrolidine derivatives, II and III, the 6-axial proton of IV should appear at higher field when *syn* to the nitrosamino O atom than when *anti*; thus P3′ is due to Hb′, while Hb gives rise to P3. The equatorial protons at the 2 and 6 positions, on the other hand, appear from molecular models to lie very close to the plane defined by the N—N=O group, and should thus be deshielded by a *syn* nitroso group: P1 and P2 must therefore be due to Ha and Hc respectively, while Ha′ and Hc′ give rise to P1′ and P2′, respectively. The shifts of all three protons upon conformational interconversion imply that the single conformer present in the crystal (Fig 3a) has the *syn* stereochemistry, as in IVa.

Confirmation of our NMR spectral assignments, and thus of the stereochemical inferences based upon them, can be found in the data of Table 4, which lists the magnitudes of the upfield shifts ($\Delta\nu$) experienced by all six low-field peaks when the solvent is changed from chloroform-d to benzene-d_6. Karabatsos and Taller[7] have shown that protons α to the nitrosamino function have larger $\Delta\nu$ values when *anti*

TABLE 4. UPFIELD SHIFTS ($\Delta\nu$) EXPERIENCED BY THE α-NITROSAMINO PROTONS OF NITROSOPIPECOLIC ACID IN AN AROMATIC SOLVENT

Peak[a]	Real position[b]		$\Delta\nu$[c]	Stereochemical assignment
	C_6D_6	$CDCl_3$		
1	4·46	4·29	0·17	Ha
1′	4·82	4·46	0·36	Ha′
2	5·67	5·22	0·45	Hc
2′	5·30	5·07	0·23	Hc
3	6·55	6·14	0·41	Hb
3′	7·56	7·20	0·36	Hb′

[a] Peaks are numbered as in Figure 3c.

[b] The peak positions given represent centers of density, rather than actual chemical shifts; values are given in τ units.

[c] $\Delta\nu = \delta C_6D_6 - \delta CDCl_3$; $\Delta\nu$ values are given in p.p.m.

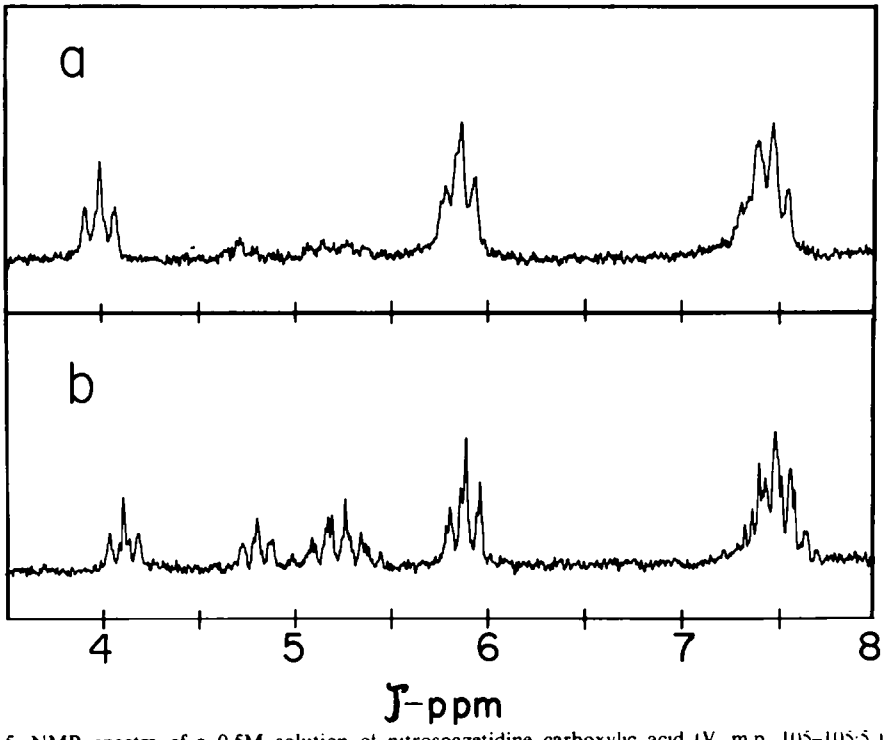


FIG 5. NMR spectra of a 0·5M solution of nitrosoazetidine carboxylic acid (V, m.p. 105–105·5°) in pyridine-d_5 containing TMS as internal reference: (a) upon dissolution, at −40°, (b) 65 min after raising the temperature to 0°.

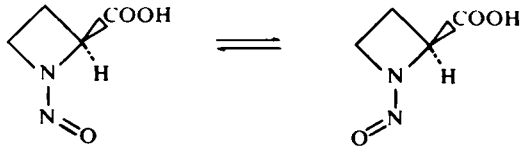
to the oxygen atom than when *syn*, and they have concluded that this effect, which they attribute to stereospecific association between the nitrosamino group and the benzene ring, "can be used as a reliable criterion of assigning configurations". The stereochemical assignments that follow from the significant differences in $\Delta\nu$ values within each pair of resonances listed in Table 4 are those in the preceding paragraph.

The inference that the bulky carboxyl group at the 2-position of nitrosopipecolic acid prefers the axial orientation (*vide supra*) is an important corollary of these stereochemical assignments. That this preference is marked is suggested by comparison of the $\Delta\delta$ values[7] for the α-nitrosamino protons of the heterocycles II–V. The $\Delta\delta$ value for Hb (1·05 ppm) is considerably larger than any of those (0·60 ± 0·05 ppm) for the α-nitrosamino protons of compounds II, III, and V (Figs 1, 2, and 5), suggesting that the dihedral angle defined by the Hb—C—N—N system in IV must be substantially greater than the corresponding angles (each of which is undoubtedly at least 60°) for compounds II, III, and V. As mentioned previously, the negative $\Delta\delta$ value (−0·30 ppm) for Hc implies that the Hc—C—N—N dihedral angle is very nearly 0°, on the average. The relative insensitivity of peak shape and other spectral details to changes in solvent (*cf* Figs 3c and 4a) indicates that the axial orientation is preferred in both polar and non-polar media, and the close similarity of the widths at half-height of P1 and P1′ demonstrates that the preference for the axial position persists even in the *anti* conformer, IVb.

These initially rather surprising observations appear to be unexceptional. Harris and Spragg,[11] and Chow, *et al.*[12] have reported similar behavior among a variety of heterocyclic nitrosamines. As a striking illustration of the potent orienting influence of the nitrosamino group upon α-substituents, these authors have shown that even *cis*-2,6-dimethyl-1-nitrosopiperidine preferentially assumes a conformation in which both Me groups are axial.

The origin of the one-proton multiplet at 7·40–7·70 τ in the NMR spectra of IV is not certain, but the apparent spectral change resulting from deuteration at the 2 position (Fig 4) together with the fact that it is shifted toward slightly lower field in IVb, has led us to the tentative conclusion that one of the 3-protons is responsible. Its appearance nearly 1 ppm downfield from the other five C—CH_2—C protons was unexpected, and may reflect a pronounced dependence of the carboxyl group's deshielding influence upon dihedral angle.

In contrast to the four nitrosamino acids described above, nitrosoazetidine-carboxylic acid appears to crystallize preferentially in the *anti* conformation, Vb. Fig 5 shows that the conformer which appears in solution upon standing has methinyl and α-methylene resonances at higher and lower field, respectively, than those of the conformer which predominates in the crystal. Using arguments essentially the same as those upon which the spectral assignments for the pyrrolidine derivatives, II and III, were based, we have attributed the resonances at 4·00–4·20 and 5·75–5·97 τ to



Va Vb

the 2-methinyl and 4-methylene protons, respectively, of Vb, and the patterns at 4·70–4·90 and 5·00–5·45 τ to the corresponding protons of Va.

Initial attempts to determine the conformation of crystalline nitrosoazetidinecarboxylic acid were complicated by the fact that the energy barrier to conformer interconversion is substantially smaller in V than in any of the other nitrosamino acids. The half-life for the *anti* ⇌ *syn* interconversion is only 10–15 min at 0° in pyridine-d_5, and it was necessary to work rapidly at temperatures just above the m.p. of the solvent to obtain reliable information on the conformational picture in the crystalline material.

A second illustration of the low barrier to rotation about the N–N bond in this molecule is found in the data collected at higher temperatures. All of the signals in the NMR spectrum of V are greatly (but reversibly) broadened upon heating pyridine-d_5 solutions to 110°, while the spectra of compounds I–IV are at least as well defined under these conditions as they are at room temperature.

EXPERIMENTAL

All of the amino acids were obtained from Aldrich Chemical Co., Milwaukee, Wisc. (except as otherwise indicated), and were used without further purification. Mass spectra were taken on an AEI-MS-9 instrument; all of the materials were inserted with a direct inlet probe. NMR spectra were obtained with a Varian HA-100 spectrometer. UV absorption spectra were taken on a Cary Model 15 spectrophotometer and IR spectra were determined on a Beckman IR-18. Optical rotations were determined for the aqueous solns using a Perkin–Elmer Model 141 polarimeter. Analyses were carried out by Micro-Tech Laboratories, Skokie, Illinois. M.ps were determined in capillary tubes and are corrected. p*Ka* values were determined by titrating a 0·01M aqueous soln of each acid against an equal volume of 0·01M NaOH; the p*Ka* was taken to be the pH at the half-neutralization point.

N-*Nitrososarcosine* (I)

An aqueous soln of $NaNO_2$ (55·2g) was added over a period of 40 min to an ice cold, magnetically stirring soln of 67 ml conc HCl and 35·6g sarcosine in 100 ml water. The mixture was stirred in the cold for 1 hr after addition was complete. The water was evaporated under reduced pressure, and the residue was extracted with 400 ml acetone. The acetone soln was filtered and evaporated, and the oily residue (34g, 72%) was allowed to crystallize. Repeated recrystallization from chloroform or EtOAc gave a pale yellow sample, m.p. 66–67°. The p*Ka* value of 3·2, which was determined using a freshly prepared soln, was found to be invariant with time, showing that any difference in p*Ka* between conformers Ia and Ib is smaller than the experimental error: IR spectrum in chloroform: ν_{max} 1455 cm^{-1} (NO), 1735 cm^{-1} (CO). Found: C, 30·25; H, 5·07; N, 23·86. Calc. for $C_3H_6N_2O_3$: C, 30·51; H, 5·12; N, 23·72%).

Mass spectrum: parent at *m/e* 118; fragments at *m/e* 88 (loss of NO) and *m/e* 73 (loss of COOH).

N-*Nitroso*-L-*azetidinecarboxylic acid* (V)

L-azetidine-2-carboxylic acid (100 mg) was dissolved in water (10 ml) and 2N H_2SO_4 (1·5 ml); the soln was cooled in ice. $NaNO_2$ (0·25g) was added a little at a time during 15 to 20 min, and the mixture was allowed to react for 1 hr. After standing at room temp for a few min, the aqueous soln was extracted 7 times with 40 ml ether and the extract was evaporated to dryness at room temp in a stream of N_2. The residue weighed 102 mg, and was crystallized from ether by addition of methylene chloride and cooling, yield: 66 mg, colorless crystals, m.p. 106–107° (dec); p*Ka* = 2·8; $[\alpha]_D^{25} = -335°$ ($c = 0·22$ in water); ν_{max} (halocarbon oil mull) 1400 cm^{-1} (NO) and 1720 cm^{-1} (CO). (Found: C, 36·66; H, 4·63; N, 21·31. Calc. for $C_4H_6N_2O_3$: C, 36·90; H, 4·65; N, 21·53%).

Mass spectrum: parent peak at 130·0388 amu; $C_4H_6N_2O_3$ = 130·0378. Fragments at *m/e* 100 (loss of NO), 85 (loss of COOH) and 55 (loss of COOH, NO).

Attempts to crystallize the nitroso acid from hot EtOH gave an uncrystallizable oil, identified as the ethyl ester by mass spectrometry: parent peak at 158·0696 amu; calculated for $C_6H_{10}N_2O_3$ = 158·0691. Fragmentation was as for the acid, except for loss of $COOC_2H_5$ (fragments at *m/e* 128, 85 and 55).

Decarboxylation. To a few mg of the acid in 2 ml water was added 1 drop 20% NaOH. The soln was warmed for a few min on a steam bath and allowed to stand at room temp overnight. One drop of the soln was examined in the mass spectrometer. No parent at *m/e* 130, corresponding to the acid, was seen. There was a major peak at 86·0481 amu which was identified as nitrosoazetidine, $C_3H_6N_2O$ = 86·0480; a fragment of mass 56·0499 corresponded to C_3H_6N = 56·0500; this is a normal fragment seen in the mass spectrum of nitrosoazetidine under the same conditions.

N-*Nitroso*-L-*proline* (III)

Proline (30g) was dissolved in HCl (20 ml) and water (100 ml) cooled in ice, and $NaNO_2$ (25g) was added slowly. After reacting 1 hr, the water was evaporated from the nitrosation reaction using a rotary evaporator and the nitrosoproline was extracted with pure acetone. The acetone was removed by evaporation in the cold in a stream of N_2. The first crop of crystals which appeared weighed 16·8g, and was crystallized from chloroform, m.p. 99–100°. The second crop weighed 2·6g, m.p. 98·5–99°. Both were colorless. The NMR spectra indicated that both crops contained only N-nitrosoproline. A third crop was crystallized from chloroform and weighed 6g, m.p. 97·5–98°.*

Recrystallization from benzene gave pale yellow crystals, m.p. 100–101° (dec); p*Ka* = 3·0 ν_{max} (in chloroform) 1430 cm^{-1} (NO) and 1730 cm^{-1} (CO); $[\alpha]_D^{25} = -185°$ (*c* = 0·23 in water).

(Found: C, 41·65; H, 5·59; N, 19·48. Calc. for $C_5H_8N_2O_3$: C, 41·55; H, 5·55; N, 19·45%).

Mass spectrum: parent peak at 144·0537 amu; $C_5H_8N_2O_3$ = 144·0535. Fragments at *m/e* 99 (loss of COOH) and 69 (loss of COOH, NO).

Decarboxylation. 50 mg of nitroso-L-proline was dissolved in 5 ml water. 2 drops of 20% NaOH were added and the flask was heated in hot water for several min and allowed to stand overnight. A smell of nitrosamine was apparent. One drop of the soln was examined in the mass spectrometer. There was no parent at *m/e* 144. The apparent parent was at *m/e* 100 and was 100·0638; $C_4H_8N_2O$ (nitrosopyrrolidine) = 100·0637. The characteristic fragmentation of nitrosopyrrolidine was seen, with a major fragment at 69·0578; C_4H_7N = 69·0578.

Nitroso-DL-*proline*

On allowing an aqueous soln of nitroso-L-proline containing 6g/100 ml to stand in the cold for more than a week, light red crystals appeared. Examination of these crystals showed them to be nitrosoproline (the mass spectrum and NMR spectrum were identical with those described). (Found: C, 41·84; H, 5·67; N, 19·42. Calculated for $C_5H_8N_2O_3$: C, 41·55; H, 5·55; N, 19·45%). The crystals melted at 114–115° and were optically inactive.

N-*Nitroso*-4-*hydroxy*-L-*proline*

Hydroxyproline (30g) was dissolved in 30 ml 10N HCl and 110 ml water, and cooled in ice. $NaNO_2$ (25g) was added slowly and the compounds were allowed to react for 1 hr. The soln was evaporated to dryness at 50°, using a rotary evaporator. Above the inorganic material was a pale yellow oil, which crystallized on cooling. The residue was extracted with approximately 200 ml warm acetone in several portions and the insoluble inorganic compounds were filtered off. The acetone was evaporated in a stream of N_2. The oily semisolid was filtered off, washed with a little cold acetone and dried in air. The yield was 13·5g of colorless crystals, m.p. 114–115°. From the mother liquor was obtained a second crop of 6g, m.p. 115–115·5 , which was unchanged by recrystallization from acetone, p*Ka* = 2·90; ν_{max} (in halocarbon oil mull) 1450 cm^{-1} (NO) and 1750 cm^{-1} (CO); $[\alpha]_D^{25} = -192°$ (*c* = 0·26 in water). (Found: C, 37·37; H, 5·05; N, 17·41. Calculated for $C_5H_8N_2O_4$: C, 37·45; H, 5·00; N, 17·49%).

Mass spectrum; parent peak at 160·04/8 amu; $C_5H_8N_2O_4$ = 160·0484. Fragments at *m/e* 130 (loss of NO), 115 (loss of COOH) and 80 (loss of COOH, OH, H_2O).

Decarboxylation. 50 mg of nitrosohydroxyproline was dissolved in 0·5 ml water and 2 drops 20% NaOH were added. The soln was warmed for a few min in hot water and allowed to stand overnight. One drop of

* Earlier preparations in which the nitrosoproline was recrystallized from MeOH or from technical acetone (containing isopropanol) led to conversion of the nitrosoproline into the methyl and isopropyl esters, respectively. The methyl ester was hydrolyzed by addition of 65% NaOH in water. After standing overnight the equivalent of HCl was added and the volatile material was evaporated. The residue was extracted with acetone, which was then evaporated, and the nitrosoproline was crystallized from benzene giving red-brown crystals, m.p. 106·5–107·5° (Found: C, 41·42; H, 5·54; N, 19·48).

the soln was examined in the mass spectrometer. There was no parent at *m/e* 160. The apparent parent had a mass of 116·0587; $C_4H_8N_2O_2$ (nitrosohydroxypyrrolidine) = 116·0586. A fragment was present at *m/e* 99, corresponding to loss of OH, and a further fragment at *m/e* 69, corresponding to loss of OH, NO.

N-*Nitroso*-DL-*pipecolic acid* (N-*nitrosopiperidine*-2-*carboxylic acid*)

Racemic pipecolic acid (3·04 g) was dissolved in 30 ml N HCl and cooled in ice. $NaNO_2$ (2·2 g) was added slowly and the mixture stood for 1 hr. The soln was extracted with 3 × 15 ml methylene chloride, the combined extracts were dried over Na_2SO_4 and the solvent was removed with N_2. The residual solid weighed 2·96 g. The compound was crystallized from benzene. The first crop of colorless crystals weighed 0·87 g, m.p. 91–93°, and after crystallization from benzene weighed 546 mg, m.p. 91–92°. A second crop weighed 1 g and, after recrystallization from benzene, 596 mg. The colorless crystals had m.p. 91–92° (dec),* p*Ka* = 3·0; ν_{max} (in chloroform) 1455 cm^{-1} (NO) and 1730 cm^{-1} (CO). Found: C, 45·30; H, 6·45; N, 17·66. Calc. for $C_6H_{10}N_2O_3$; C, 45·50; H, 6·33; N, 17·71%).

Mass spectrum: parent peak at 158·0695 amu; $C_6H_{10}N_2O_3$ = 158·0692. Fragments at *m/e* 128 (loss of NO), 113 (loss of COOH), 83 (loss of COOH, NO).

Decarboxylation. Racemic nitrosopipecolic acid (50 ml) was dissolved in 0·5 ml distilled water and 2 drops 20% NaOH were added. The soln was warmed in hot water and allowed to stand for several hr. One drop of the soln was examined in the mass spectrometer. The mass spectrum showed a small parent at *m/e* 158 (unchanged acid), a larger peak at *m/e* 156 and a large peak at *m/e* 114. This latter peak had a mass of 114·083 amu; $C_5H_{10}N_2O$ (nitrosopiperidine) = 114·079.

N-*Nitroso*-L-*pipecolic acid*

Racemic nitrosopipecolic acid (1·3 g) was dissolved in 50 ml hot acetone, 2·68 g quinine was added and the clear soln was cooled. The crystals which separated were filtered off, and washed with cold acetone, yielding 2·8 g, m.p. 158–159·5°. The solid was recrystallized from hot acetone (in which it would not dissolve completely). The yield was 1·4 g, m.p. 173–173·5°. The quinine salt (1·3 g) was suspended in 50 ml water and approximately 5 ml 2 N HCl was added. The soln was extracted 3 times with 50 ml methylene chloride, the extracts were dried with $NaSO_4$ and the solvent was removed in a stream of N_2. 360 mg of oil remained, which later crystallized. After crystallization from benzene the yield was 200 mg of colorless crystals, m.p. 105–105·5°, $[\alpha]_D^{25} = -187°$ (*c* = 0·23 in water).

The D-isomer could not be obtained in a pure state, although by crystallization of the mother liquor of the quinine salt of the nitroso acid a crop of crystals was obtained and discarded and the remaining mother liquor was evaporated to dryness, leaving a gelatinous material, which set to a pale yellow solid, m.p. m.p. 147·5–149°. This material (1·3 g) was acidified and extracted exactly as above. After evaporation of the methylene chloride, 462 mg of pale yellow oil remained which crystallized considerably more slowly than the L-isomer. After crystallization from benzene (it was much more soluble than the L-acid), 230 mg of colorless crystals were obtained, m.p. 94·5–95·5°, $[\alpha]_D^{25} = +85°$ (*c* = 0·30 in water).

Denitrosation. Chloroform solns of both the L- and D- acids, after standing for 2 weeks at room temp, deposited colorless crystals, which were found, by mass spectrometry,* to be pipecolic acid. No explanation of the mechanism of this denitrosation can be given.

Nitrosopipecolic acid-2-d

Racemic nitrosopipecolic acid (155 mg) was dissolved in 1 ml 7·5 M NaOD in D_2O. Three drops of t-BuOH was added as an internal reference and the NMR spectrum was determined. After standing at room temp overnight, the low-field resonance had disappeared; the soln was acidified and extracted with CH_2Cl_2. An NMR spectrum in chloroform-d soln is shown in Fig 4b. Final confirmation of the structure was obtained from the mass spectrum, which showed a parent ion at *m/e* 159 with fragments at *m/e* 129, 114, and 84.

* A larger scale preparation of nitrosopipecolic acid used 21g of pipecolic acid, which was dissolved in 125 ml SN HCl cooled in ice. 20g $NaNO_2$ was added slowly during 1 hr; the soln. was extracted with 3 × 100 ml benzene and the solvent was removed in a stream of N_2. The remaining solid was crystallized from benzene, yielding 23g of colorless crystals, m.p. 89–90°.

* Parent at 129·0788 amu; $C_6H_{11}NO_2$ = 129·0789, fragment at *m/e* 84 (loss of COOH).

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