Articles

Discodermide: A New Bioactive Macrocyclic Lactam from the Marine Sponge *Discodermia dissoluta*

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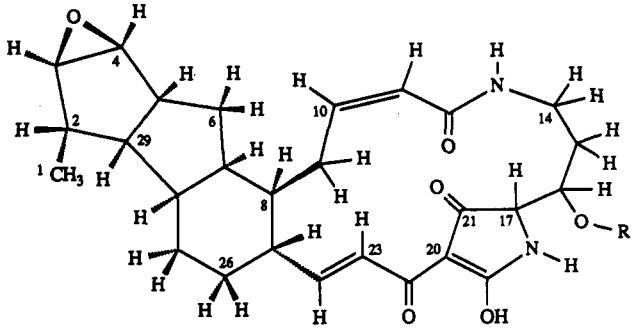
A new macrocyclic lactam, discodermide (1), was isolated from a Caribbean marine sponge, *Discodermia dissoluta*. Its structure was elucidated through a combination of spectroscopic techniques, in particular NMR spectroscopy. The carbon skeleton displayed by discodermide is new; discodermide is antifungal and cytotoxic.

The genus *Discodermia* has been previously recognized as a source of several biological agents, calyculins A–D[1] and discodermins A–D.[2] In our search for biologically active substances from marine organisms, an extract from the marine sponge *D. dissoluta* was discovered to inhibit in vitro proliferation of P388 murine leukemia cells, to inhibit growth of *Candida albicans*,[3] and to be active in the two-way mixed lymphocyte culture assay.[4] We recently described the novel polyhydroxylated lactone discodermolide as the metabolite responsible for the immunosuppressive activity of the crude extract.[5] We now report the isolation and the structure determination of the cytotoxic and antifungal active compound as discodermide (1).



1 R = H
2 R = CH_3CO

The sponge, *D. dissoluta*, was collected by scuba at Lucaya, Grand Bahama Island, at a depth of 33 m. A methanol/toluene (3:1) extract was concentrated and successively partitioned between equal volumes of EtOAc/H_2O and BuOH/H_2O. The BuOH-soluble fraction inhibited the growth of *C. albicans*. Bioassay-directed chromatographic separation of this fraction resulted in the isolation of discodermide (1, 0.1% frozen wt). Discodermide (1) was obtained as a white powder (mp ~200 °C dec). The HRFAB mass spectrum indicated a molecular formula of $C_{27}H_{34}N_2O_6$. A UV absorption at λ_{max} 313 nm (ϵ 9650) indicated the presence of extended π conjugation. Infrared absorptions at 3350 and 1665 cm^{-1} revealed the presence of hydroxyl and conjugated carbonyl functionalities, respectively. The presence of an enolized β-tricarbonyl grouping was suggested by an orange-red coloration with ferric chloride and the formation of greenish yellow copper complex with cupric acetate.[6] Due to the low solubility and the overlap of proton NMR signals of 1 in CD_3OD, C_5D_5N, and $(CD_3)_2SO$, discodermide was acetylated under standard conditions and discodermide acetate 2 was used for much of the structure elucidation. Assignment of resonances in the ^1H and ^{13}C NMR spectra of 1 and 2 (Table I) were made as the result of COSY experiments, long-range COSY experiments (COSYLR and COSYRCT),[7] 2D C–H correlation experiments,[8] 2D long-range C–H correlation experiments (HMBC),[9] and decoupling experiments. The structure of the acetate was elucidated in the following manner.

The molecular formula of 2 was determined to be $C_{29}H_{36}N_2O_7$ (HRFABMS). Similar to 1, the UV spectrum of 2 exhibited an absorption indicative of an extended conjugated system [λ_{max} 312 nm (ϵ 9660)]. The IR absorptions at 1724 cm^{-1} indicated the presence of an acetate functionality, while the presence of conjugated carbonyls was suggested by a band at 1665 cm^{-1}. Resonances in the ^{13}C spectra indicated the presence of a conjugated enolized tricarbonyl system (^{13}C resonances: 192.6, 184.3, 180.65, and 102.5 ppm). The downfield signal at 192.6 ppm assigned to the enol carbon suggested that it is attached to another oxygen or nitrogen atom.[10] The presence of a conjugated amide and an acetate carbonyl were indicated by the ^{13}C NMR resonances observed at 167.3 and 170.4 ppm, respectively. Resonances in the ^1H and ^{13}C spectra of 2 indicated the presence two double bonds (^{13}C resonances observed at 125.1, 129.8, 140.3, and 147.3 ppm). These data account for 7 of the 13 degrees of unsaturation, and therefore, the carbon skeleton consists of six rings.

(1) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780. Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Koseki, K. *J. Org. Chem.* **1988**, *53*, 3930.
(2) Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron Lett.* **1984**, *25*, 5165; *Ibid.* **1985**, *26*, 855.
(3) Odds, F. C. *Candida and Candidiosis*; Leicester University Press: England, 1979.
(4) Gunasekera, S. P.; Cranick, S.; Longley, R. E. *J. Nat. Prod.* **1989**, *52*, 757.
(5) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912.
(6) Ito, S.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1813.
(7) Derome, A. *Modern NMR Techniques for Chemistry Research*; Pergamon: New York, 1987.
(8) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.
(9) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093. Bax, A.; Aszalos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056.
(10) Barrow, C. J.; Blunt, J. W.; Munro, M. H. G.; Perry, N. B. *J. Nat. Prod.* **1988**, *51*, 275.

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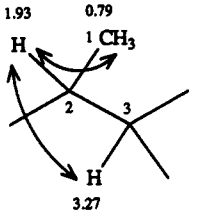
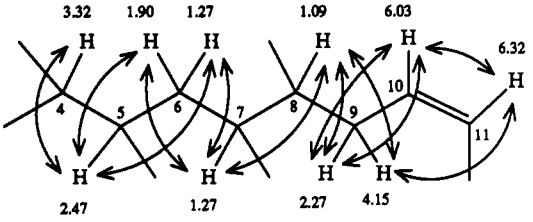
Interpretation of the data from a COSY experiment of **2** and from a series of decoupling experiments identified the partial structures C1–C3 (A), C4–C11 (B), C14–C17 (C), and C23–C29 (D). The 11.5-Hz coupling constant



A



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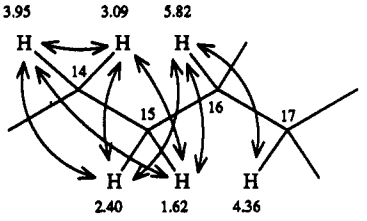

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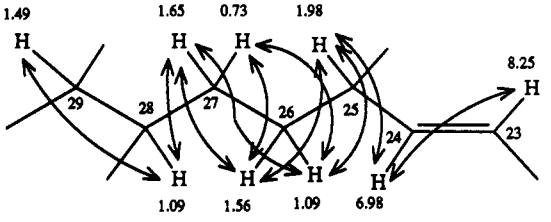
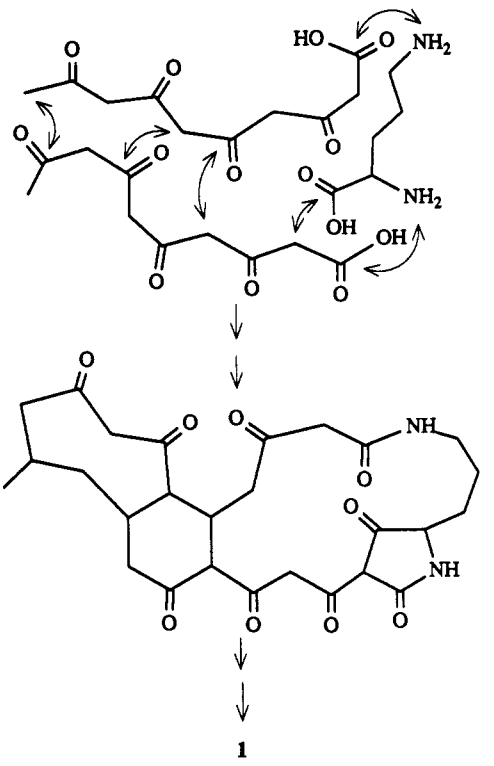


D

between H10 and H11 and the 15.3-Hz coupling constant between H23 and H24 are consistent with *Z* and *E* olefinic bonds, respectively. The COSYRCT experiment optimized for four-bond couplings indicated coupling of H2 with H4 and H3 with H5. This suggested a linkage between C3 and C4 atoms. This was confirmed by the ^{1}H-detected heteronuclear multiple bond correlation experiment (HMBC, Table I). The ^{13}C chemical shift values of 66.3 and 61.1 ppm for C3 and C4 in **2**, respectively, observed by the C–H correlation experiment suggest the attachment of oxygen atoms at these two carbons. The characteristic C–H coupling constants (C3, J_{C-H} = 180 Hz and C4, J_{C-H} = 182 Hz) suggested the presence of an epoxide ring.[11] The partial structure B H11 (6.32 ppm) and H10 (6.03 ppm) exhibited two-bond and three-bond couplings to an amide carbonyl carbon appearing at 167.3 ppm, respectively. This carbonyl resonance in turn exhibited two-bond and three-bond couplings to H13 (NH) and H14, respectively, and thus established the connectivity between C11 and C14 across the amide group. Acetylation of discodermide

Scheme I. Suggested Biosynthesis of Discodermide (1)



1

1 to give discodermide acetate **2** shifted the proton signal attributed to H16 from 4.84 to 5.82 ppm, thus confirming the location of a hydroxyl at C16 in discodermide. In the long-range C–H correlation spectrum of **2** there were cross-peaks appearing from H17 (4.36 ppm) to C19 (192.6 ppm) and C21 (180.5 ppm) and H18 (9.20 ppm) to C17 (65.1 ppm), C19, and C20 (102.5 ppm). These connectivities established the connection between the partial structure C and the cyclized β-tricarbonyl enol system. Although unusual, this is not new.[6,12] The remaining carbonyl C22 (184.3 ppm) of the β-tricarbonyl enol system indicated a long-range correlation to H23 (8.25 ppm) and H24 (6.98 ppm) and thus established the connectivity between the partial structure D and the tricarbonyl system. The downfield chemical shift value of H23 is consistent with the α,β-unsaturated carbonyl group. However, the large downfield chemical shift value of H24 could be due to the anisotropic effect caused by the adjacent β-tricarbonyl enol system. The ^{1}H COSY spectrum of **2** indicated coupling between H2 (1.93 ppm)/H29 (1.49 ppm) and H5 (2.47 ppm)/H29. The long-range C–H correlation spectrum exhibited couplings between H1/C29 and H2/C29 and H3/C29. These data established the connectivity between the partial structures A and D. The three-bond coupling between H4 and C29 suggested a linkage between C5 and C29 and thus established a five-membered ring system.

The ^{1}H COSY spectrum of **2** indicated coupling between H28 (1.09 ppm) and H7 (1.27 ppm). The ^{1}H COSYRCT spectrum indicated four-bond coupling between H5 (2.47 ppm) and H28. The long-range C–H correlation spectrum exhibited three-bond couplings between H6 (1.90 ppm)/C28, H2 (1.93 ppm)/C28, H28/C2 (36.9 ppm), H28/C8, and H27 (1.65 ppm)/C7. These data established the connectivity between C7 and C28 forming the second five-membered ring. The coupling observed between H8

(11) Kalinowski, H.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley and Sons: New York, 1988; p 499.

(12) Stikings, C. E. *Biochem. J.* **1959**, *72*, 332. Stikings, C. E.; Townsend, R. J. *Ibid.* **1961**, *78*, 412.

Table I. ^1H and ^{13}C NMR Data for Compounds 1 and 2 and the Long-Range Connectivities Observed in the HMBC Experiment

	1		2		
position	H[a]	C[b]	H[a]	C[a]	HMBC, (^1H)[a]
1	0.79	17.7	0.79 (d, 7.0)	18.2 (q, 119)	H2, H3, H29
2	1.95	36.2	1.93 (dq, 7.0, 1.8)	36.9 (d, 126)	H3, H4, H29
3	3.26	66.7	3.27 (s)	66.3 (d, 180)	H1, H2, H29
4	3.31	61.3	3.32 (s)	61.1 (d, 182)	H2, H6, H29
5	2.49	41.2	2.47 (ddd, 8.3, 8.0, 7.2)	41.9 (d, 123)	H2, H3, H4
6α	1.90	30.6	1.90 (m)	30.9 (t, 126)	
6β	1.26		1.27 (m)		
7	1.26	47.3	1.27 (m)	47.9 (d, 115)	H6, H9, H27
8	1.07	44.1	1.09 (m)	45.0 (d, 120)	H6, H10, H24
9	4.13	29.4	4.15 (ddd, 12.8, 12.0, 1.5)	29.3 (t, 124)	
9	2.31		2.27 (ddd, 12.8, 2.0, 2.0)		
10	6.03	141.3	6.03 (dd, 11.5, 7.1)	140.3 (d, 149)	H9, H11
11	6.28	123.0	6.32 (d, 11.5)	125.1 (d, 156)	H9
12		167.2		167.3 (s)	H10, H13, H14
13	9.13		9.25 (t, 5.8)		
14	4.06	36.5	3.95 (dddd, 11.0, 5.8, 5.5, 4.0)	37.4 (t, 129)	H13, H15, H16
14	3.20		3.09 (dddd, 11.0, 9.8, 5.8, 1.5)		
15	2.44	29.7	2.40 (dddd, 11.8, 9.8, 5.5, 1.0)	29.8 (t, 125)	H16, H17
15	1.60		1.62 (dddd, 11.8, 5.8, 4.0, 1.5)		
16	4.84	70.6	5.82 (ddd, 5.8, 1.0, 1.0)	75.4 (d, 145)	H14, H17
16	6.62				
17	4.40	65.5	4.36 (s)	65.1 (d, 135)	H18
18	9.02		9.20 (s)		
19		193.5		192.6 (s)	H17, H18
20		101.1		102.5 (s)	H18
21		178.5		180.5 (s)	H17
22		183.9		184.3 (s)	H23, H24
23	8.28	128.1	8.25 (d, 15.3)	129.8 (d, 160)	H25
24	6.97	147.5	6.98 (dd, 15.3, 10.3)	147.3 (d, 150)	H25
25	2.03	45.6	1.98 (m)	45.7 (d, 119)	H9, H23, H27
26α	1.49	32.2	1.56 (m)	33.2 (t, 120)	H24
26β	1.07		1.09 (m)		
27α	1.64	30.2	1.65 (m)	30.3 (t, 120)	H26, H29
27β	0.73		0.73 (m)		
28	1.07	50.8	1.09 (m)	51.4 (d, 117)	H1, H2, H6, H26, H27
29	1.45	57.5	1.49 (m)	58.2 (d, 124)	H3, H4, H27
30				170.5 (s)	H16
31			1.80 (s)	20.9 (q, 127)	

[a] Pyridine-d_5 at 50 °C. [b] CD_3OD. Chemical shifts (ppm) from solvent (multiplicity, J (Hz)).

Table II. NOEs Observed for Discodermide Acetate

^1H irradiated	NOE (%)	^1H irradiated	NOE (%)
1-CH_3	2 (4.5), 3 (4.0), 29 (2.0)	7-H	8 (2.0), 28 (3.0)
2-H	1 (3.0), 3 (0.5)	8-H	9 (1.5), 9 (1.5)
5-H	4 (6.5), 6α (2.5), 29 (6.5)	25-H	23 (4.0), 26β (2.0), 27β (1.5)
6-αH	7 (2.0), 10 (1.3)		
6-βH	4 (1.0), 6α (4), 10 (2.5)	27-H	2 (1.0), 28 (2.1)
		29-H	28 (3.0)

(1.09 ppm) and H25 (1.98 ppm) and the long-range C–H correlations observed between H26 (1.09 ppm)/C8 and H24 (6.98 ppm)/C8 confirmed the linkage between C8 and C25 thus forming the six-membered ring system, which accounted for the last degree of unsaturation in the molecule.

The relative stereochemistry of the tetracyclic ring system was established by difference NOE experiments (see Table II). The NOEs between H1/H29, H5/H29, H28/H29, H4/H5, and H5/H6 established that they are on the same side of the ring system. The protons H3 and H4 must have a cis configuration since they are part of the epoxide ring formed on a five-membered ring system. The NOEs between H6 (1.90 ppm)/H7, H7/H28, H7/H8, and H28/H27 (1.65 ppm) established the relative stereochemistry of these protons. Similarly the NOEs between H27 (0.73 ppm)/H25 and H25/H26 (1.09 ppm) completed the relative stereochemistry of the tetracyclic end of the molecule. The NOE between H27 (1.65 ppm)/H2 and H6/H10 confirmed the cis-syn-cis orientation of the cyclopent[a]octahydroindene part of the molecule. The relative stereochemistry at C16 and C17 could not be determined using NOE.

It appears likely that discodermide 1 is biosynthetically derived from ornithine and two pentaacetate chains as outlined in Scheme I, and as suggested, in ikarugamycin[6] cyclization, reduction, and hydroxylation in ring A and at the β-carbon of ornithine occur subsequently to yield 1.

Discodermide inhibits the in vitro proliferation of cultured murine P388 leukemia cells with an IC_{50} of 0.3 μg/mL. It also inhibits the growth of *C. albicans* with an MIC of 12.5 μg/mL.

Experimental Section

NMR spectra were recorded at 360 MHz for ^1H and 90.5 MHz for ^{13}C. All chemical shifts were recorded with respect to the solvent (C_5D_5N, highest field signal at 7.19 ppm for ^1H and 123.5 ppm for ^{13}C).

Collection and Extraction. The sponge *D. dissoluta* was collected from Lucaya, Grand Bahama Island, at a depth of 33 m in March 1987 and was immediately frozen. A voucher specimen is on deposit at the Harbor Branch Oceanographic Museum (catalog no. 003:00060). The freshly thawed sponge (434 g) was extracted exhaustively with a mixture of methanol and toluene (3:1). The solvent was removed in vacuo from the combined extracts. The resulting extract was partitioned between EtOAc and H_2O, and the H_2O-soluble fraction was extracted with BuOH. The BuOH soluble fraction was chromatographed on reversed-phase C_{18} with H_2O/MeOH gradient. The fraction that eluted with 10–20% H_2O/MeOH furnished the most potent *C. albicans* activity. Discodermide was crystallized from this fraction

using $MeOH/CH_2Cl_2$ (3:2), 115 mg (0.1% from frozen sponge).

Discodermide (1): mp ~200 °C dec; $[\alpha]_D$ 97.5° (c 0.2, $CHCl_3$/MeOH (1:1); UV λ_{max} (MeOH) 313 (ε 9650), 238 (16 500) nm; IR (KBr) 3350, 2910, 2460, 1665, 1600, 1465, 1227, 995, 840 cm^{-1}; ^1H and ^{13}C NMR, Table I; HRFABMS (Thio) m/z 505.2312, Δ 0.7 μm for $C_{27}H_{34}N_2O_6Na$ (MNa^+); LRFABMS (Thio) m/z (relative intensity) 505 (58), 313 (17), 239 (23), 217 (100), 181 (34).

Acetylation of Discodermide. A solution of discodermide (25 mg) in pyridine (2 mL) and acetic anhydride (0.5 mL) was stirred overnight. The solvents were removed in vacuo and the resulting gum was purified on a reversed-phase amino Sep-Pak with 20% H_2O/MeOH to give discodermide acetate, 22 mg.

Discodermide acetate (2): colorless gum; $[\alpha]_D$ 77.5° (c 0.17, MeOH); UV λ_{max} (MeOH) 312 (ε 9660), 238 (16 500) nm; IR (KBr) 3360, 2900, 2500, 1724, 1655, 1600, 1465, 1240 cm^{-1}; ^1H and ^{13}C NMR, Table I; HRFABMS (mb) m/z 547.2304, Δ 0.9 μm for $C_{29}H_{36}N_2O_7Na$ (MNa^+); LRFABMS (Thio) m/z (relative intensity) 547 (100, MNa^+), 525 (25, MH^+), 505 (20), 487 (55), 469 (15), 429 (18), 319 (34).

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Supplementary Material Available: ^1H and ^{13}C NMR spectra, COSYRCT, COSY, C-H correlations, HMBC, and NOE experimental reference spectrum for 2 (17 pages). Ordering information is given on any current masthead page.

Chemistry of *N*-Nitroso Compounds. 1. Synthesis and Stereodynamics of *N*-Nitrosopiperidines and *N*-Nitrosopiperidin-4-ones

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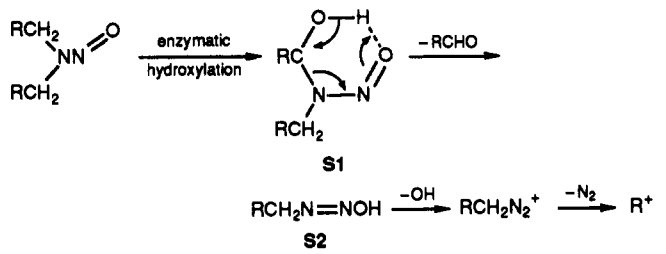
The stereochemistry of a series of *N*-nitroso-*r*-2,*c*-6-diphenylpiperidin-4-ones (**10–15**) and *N*-nitroso-*r*-2,*c*-6-diphenylpiperidines (**16–18**) in solution has been investigated by ^1H NMR, ^{13}C NMR, and dynamic ^1H NMR spectroscopic studies. Unlike the *r*-2,*c*-6-dialkyl-*N*-nitrosopiperidines, which have diaxial alkyl groups, these *N*-nitroso-*r*-2,*c*-6-diphenylpiperidines (**16–18**) and *N*-nitroso-*r*-2,*c*-6-diphenylpiperidin-4-ones (**10–15**) have diequatorial phenyl groups even though the nitroso group is coplanar to the dynamically averaged plane of the piperidine ring. The rotamer populations of the N–NO orientations in the nitrosamines were derived from continuous wave ^1H NMR spectral data and are correlated with the steric bulk of the substituents present at the C-3 and C-5 positions. *N*-Nitroso-*r*-2,*c*-6-diphenylpiperidine-4-one (**10**) was found to have a higher rotational barrier than *N*-nitroso-*r*-2,*c*-6-diphenylpiperidine (**16**). We attribute this difference to a greater degree of coplanarity (C_3–C_2–N_1–C_6–C_5) in **10** than in **16**. The rotational barriers in **10** were found to decrease as the bulk of the substituents at C_3 and C_5 increased. The rotational barrier in *N*-nitroso-*r*-2,*c*-6-diphenylpiperidine (**16**) was found to be lower than the reported rotational barriers of *N*-nitroso-*r*-2,*c*-6-dimethylpiperidine and *N*-nitroso-2,2,6,6-tetramethylpiperidine.

Introduction

Many nitrosamines are known to be carcinogenic.[1] It has also been shown that blocking of the positions α to the ring nitrogen atom by methyl groups in cyclic nitrosamines reduces the carcinogenic activity.[2] The alkyl cation formed from the nitrosamine (Scheme I) is believed to be responsible for the initial step in the process of carcinogenesis by alkylating cellular components and DNA bases.[3,4] The first step in the formation of this alkyl cation involves the enzymatic hydroxylation of an α carbon to give **S1** (Scheme I). The hydroxylation also appears to depend on the acidity of the α proton. Thus, in the case of *N*-nitrosonornicotine, where the sterically free methylenic

Scheme I



α-position is available for α-hydroxylation, the hydroxylation actually occurs at the more hindered but more acidic C-2 α-position.[5] This acidity, in turn, may also depend on the extent of electron delocalization in the N–N–O group.[6] The second step in the process leading to the

(1) (a) Magee, P. N.; Montesano, R.; Preussmann, R. *Chemical Carcinogens*; American Chemical Society: Washington, DC, 1976; p 491. (b) Ferguson, L. N. *Chem. Soc. Rev.* **1975**, *4*, 289. (c) Loeppky, R. N.; Tomasik, W.; Kerride, B. E. *Carcinogenesis* **1987**, *8*, 941. (d) Loeppky, R. N.; Christiansen, R. *Environmental Aspects of N-nitroso Compounds*; IARC Sci. Publ., 1978; Vol. 19, p 117. (e) Loeppky, R. N.; Outram, J. R. *N-Nitroso Compounds: Occurence and Biological Effects*; IARC Sci. Publ., 1982; Vol. 41, p 459.

(2) Lijinsky, W.; Taylor, H. W. *Int. J. Cancer* **1975**, *16*, 318.

(3) (a) Michejda, C. J.; Kroeger-Koepke, M. B.; Koepke, S. R.; Kupper, J. *N-Nitrosamines*; American Chemical Society: Washington, DC, 1979; Chapter 5. (b) Mochisuki, M.; Tanaka, T. *Eisei Kagaku* **1978**, *24*, 271.

(4) (a) Magee, P. N.; Barnes, J. M. *Cancer Res.* **1967**, *10*, 163. (b) Ioannides, C.; Parke, D. V. *Chem. Ind. (London)* **1980**, 854.

(5) Murphy, S. E.; Heiblum, R. *Carcinogenesis* **1990**, *11*, 1663.

(6) It is noted that of the several NX=Y systems known including *N*-acylamines, *N*-ketiminylamines, and *N*-sulfinylhydrazines, only the *N*-nitroso compounds are known to be carcinogenic. It is also the case that the *N*-nitroso compounds have significantly higher rotational barriers (ca. 20–25 kcal/mol) than do members of these other groups (barriers of ca. 10–15 kcal/mol). The extensive delocalization in the *N*-nitroso compounds is responsible for the higher barriers. At the same time this delocalization would be expected to increase the acidity of the α hydrogens.

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