MESOIONIC 1,3,4-OXADIAZOLIUM-2-AMINIDE AND 1,3,4-OXADIAZOLIUM-2-OLATE: SYNTHESIS, GEOMETRY, ELECTRONIC STRUCTURE AND ANTIBIOTIC ACTIVITY

C. A. Montanari*,[a,d] A. M. Giesbrecht,[c] J. P. B. Sandall,[b] Y. Miyata,[d] and J. Miller*,[d,e]

a Departamento de Quimica, ICEx/UFMG, Campus da Pampulha, 31270-901, Belo Horizonte-MG, Brasil
b Department of Chemistry, University of Exeter, U.K.
c Instituto de Ciencias Biomedicas - USP
d Instituto de Quimica - USP
e Laboratorio de Tecnologia Farmaceutica/CCEN/UFPB - Campus I, Caixa Postal 5009 58.051-970 - João Pessoa - Paraíba - Brasil

Abstract: Six new mesoionic 1,3,4-oxadiazolium-2-aminides and 1,3,4-oxadiazolium-2-olates were synthesized and their geometry and electronic structures calculated by the MNDO-PM3 method. An alternative route for the preparation of 1,3,4-oxadiazolium-2-aminides is presented. It involves cyclodesulphurization of acylthiosemicarbazides by yellow mercuric oxide. The antimicrobial activity of the compounds against some bacteria and fungi was also evaluated.

Introduction

Mesoionic compounds are of interest not only for their chemistry but also for the range of pharmacological properties which have been verified for various members of this class of heterocyclic compounds (1-7). The synthesis of various analogues is important in gauging their potential as a source of chemotherapeutics. Thus, pursuing our on-going program on the synthesis of mesoionic compounds (8-15), we report the preparation of mesoionic 1,3,4-oxadiazolium-2-aminides (isosydnonimines) through an alternative route. Mesoionic 1,3,4-oxadiazolium-2-olates (isosydnones) were also prepared and the structures and antibiotic properties of both types of compounds were studied.

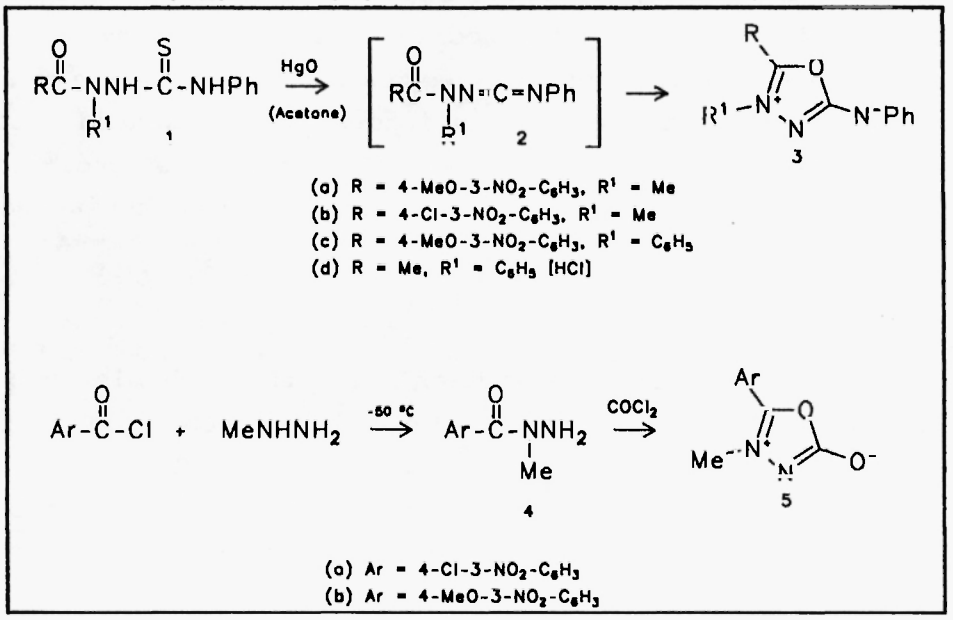
Results and discussion

Isosydnonimines were first synthesized in 1971 by the reaction of N-methyl-N-benzoylhydrazine with arylisocyanide dichlorides (16). Bicyclic isosydnonimines were prepared through the reaction of N-amino-2-pyridones

with triphenylphosphine dibromide and arylisocyanates or arylisothiocyanates. Deoxygenation of N-semicarbazide-2-pyridones with triphenylphosphine also yielded the bicyclic derivatives (17).

The formation of isosydnonimine rings is assumed to proceed by an intramolecular cyclization via carbodiimide-like intermediate valence tautomers which results in condensation cyclization (3). In this work we have similarly developed an alternative route for preparation of 1,3,4-oxadiazolium-2-aminides in good yields consisting of cyclodesulphurization of the appropriate acylthiosemicarbazide with yellow mercuric oxide, as outlined in Figure 1. The isosydnones were prepared by the reaction of 1-acyl-1-alkylhydrazines with phosgene (18, 19), according to Figure 1. The structures of 1,3,4-oxadiazolium-2-aminides, 3a-d, and 1,3,4-oxadiazolium-2-olates, 5a and b, were established by means of IR, UV, ^{1}H NMR and elemental analysis.

Figure 1: Reaction schemes for mesoionic 1,3,4-oxadiazolium-2-aminides, 3, and 1,3,4-oxadiazolium-2-olates, 5



The spectral assignments are consistent with those described for 1,3,4-oxadiazolium compounds (16). The infrared spectra show absorptions at 1630 cm^{-1} which may be assigned to C=N stretching. The NMR spectra of 3a, b show the methyl group at δ 3.67-3.94 whereas in 3d it is at δ 3.3. The u.v. and visible spectra show only one band at about λ_{max} 240-255 nm. ϵ is fairly high for these compounds, nearly 10,000, but for 1,3,4-oxadiazolium-2-aminides, 3, they are still higher, ϵ 20,000. The characteristic band for mesoionic systems (ν_{max} *ca.* 325 nm) has apparently fused with phenyl bands.

Ollis and co-workers (16) have used the absence of the carbodiimide

band at ν_{max} 2130-2155 cm^{-1} as evidence for the formation of some mesoionic ring systems. Likewise, there is no such stretching in the spectra we have obtained so far.

The infrared spectra of 1,3,4-oxadiazolium-2-olates, **5**, show bands in the region of 1750-1760 cm^{-1}, which may be assigned to C=O stretching. The u.v. spectrum of compound **5a** shows only a band λ_{max} 249 nm, with ϵ_{max} 19,686 whereas **5b** values are λ_{max} 255 nm and ϵ_{max} 16,566.

The electronic structures of 1,3,4-oxadiazolium-2-aminides, **3**, and 1,3,4-oxadiazolium-2-olates, **5**, have been calculated by means of the MNDO-PM3 (20) method using a CRAY XMP-28 computer. The bonding (and bond orders) and angles in the mesoionic compounds for which valence structures cannot be written without formal positive and negative charges can be found in Tables 1 and 2. The perspective drawings of parent molecules are displayed in Figure 2.

Table 1: Bond lengths (Å) and (bond orders) for compounds **3** and **5**

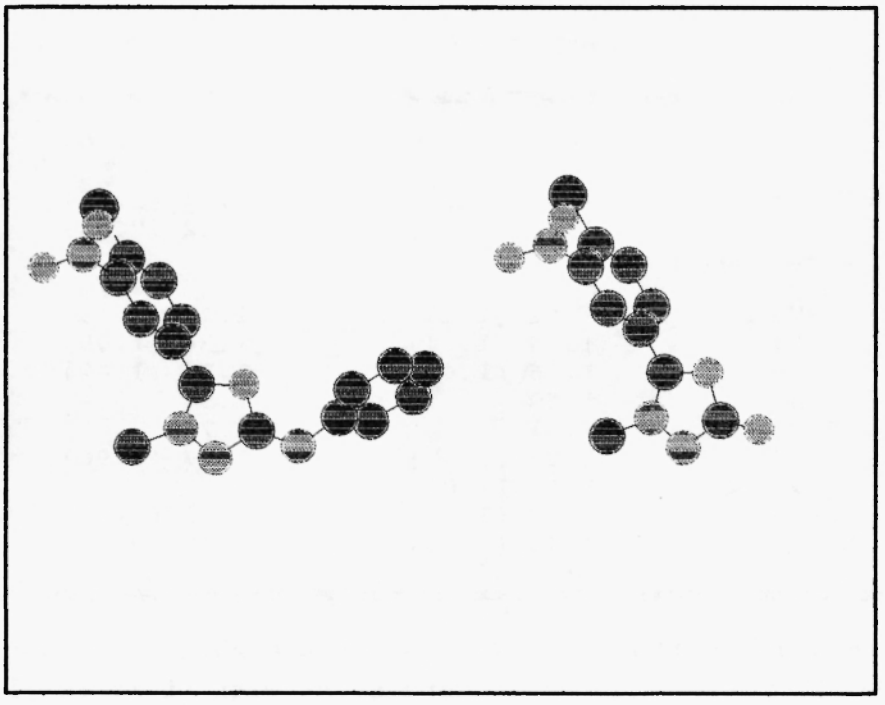
Bond	3b	5a
C5-O1	1.36 (1.09)	1.34 (1.15)
O1-C2	1.40 (0.91)	1.42 (0.83)
C2-N(exo)	1.29 (1.69)	-
C2-O	-	1.22 (1.79)
C2-N3	1.41 (1.13)	1.40 (1.15)
N3-N4	1.33 (1.14)	1.34 (1.09)
N4-C5	1.36 (1.41)	1.36 (1.42)
$=C-NO_2$	1.50	1.50
=C-Cl	1.74	1.74
=C-C5	1.47 (0.97)	1.47 (0.96)
N(exo)-C=	1.41 (1.01)	-
=C-C=	1.42 (1.42)	1.41 (1.40)
$N4-CH_3$	1.48 (0.86)	1.48 (0.87)

Analyzing the bond lengths of model compound **3b**, it is seen that the exocyclic C-N distance (1.29 Å) is consistent with those for imines (1.28 Å); for C2-N3 the bond length value (1.41 Å), is midway between values for formamide (1.36 Å) and methylamine (1.47 Å); however, it is far from those of oxadiazoles (1.33 Å). N3-N4 bond distances (1.33 Å) lie between single and double bonds as for oxadiazoles (1.29 Å). For C5-N4 and C5-O1 the bond length values (1.36 Å) are slightly higher than those for oxadiazoles (1.35 Å and 1.30 Å).

Table 2: Bond angles (°) for compounds 3 and 5

Angle	3b	5a
=C-C5-O1	120.73	120.14
C5-O1-C2	120.63	122.38
O1-C2-N(exo)	126.47	-
O1-C2-O(exo)	-	119.12
C2-N(exo)-C=	123.89	-
O1-C2-N3	106.96	107.41
C2-N3-N4	107.91	107.23
N3-N4-C5	105.52	105.61
H_3C-N4-C5	128.90	128.60
=C-C-C=	120.27	120.18
=C-C-NO_2	118.32	118.63
=C-C-Cl	121.64	121.46

Figure 2: Perspective drawings of mesoionic systems 3 and 5

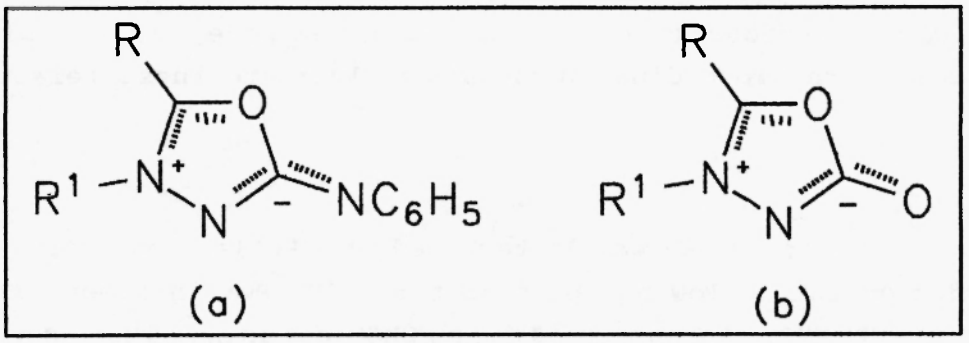


Neither bond lengths nor dihedral angles indicate distortion of the mesoionic ring, for which an essentially planar configuration is evident. Of particular interest is the aryl group attached to C-5 where the difference in dihedral angle is 66°. This could be due to an intramolecular interaction between the aryl group and the methyl group at N-4.

The present data suggest that mesoionic 1,3,4-oxadiazolium-2-aminides, 3, are better represented by the dipolar structure shown in Figure 3a, a

betaine-like structure. The calculated dipole moments for these molecules (ca. 5.36 D) support this formulation. This is consistent with the crystallographic study we have carried out with a mesoionic 1,3,4-thiadiazolium-2-aminide (21), thus supporting the theoretical study presented in this work.

Figure 3: Geometric and π-electronic structures of mesoionic rings



Similar conclusions can be drawn when the bond lengths and the bond angles of model compound 5a are considered. The calculated dipole moment is about 6.73 D this being consistent with the dipolar structure represented in Figure 3b.

Compounds 3a, d and 5a, b were assayed, by the classical agar diffusion plate method, for antimicrobial properties against some bacteria and fungi. The isosydnones 5a, b were completely inactive. In contrast, isosydnonimines 3a, d showed activity against all the microorganisms studied. The minimal inhibitory concentrations (MIC) of the active compounds are presented in Table 3.

Table 3: Minimal inhibitory concentrations (μg/ml) of isosydnonimines

Microorganism	Compound 3a	3d
A. parasiticus	250	62.5
M. canis	125	-
C. albicans	125	125
C. tropicalis	125	125
C. neoformans	125	125
R. rubra	125	250
T. glabrata	125	125
B. cereus	15	30
E. coli	30	30
P. aeruginosa	7.5	15
S. aureus	15	15
S. epidermidis	5	5

(-) Not assayed

While all the active compounds presented similar biological responses towards the microorganisms assayed, it is remarkable that the MIC values for *P. aeruginosa*, *S. aureus* and *S. epidermidis*, are very low. The high level of activity against *A. parasiticus*, which produces aflatoxins (22), is especially interesting in view of the paucity of compounds with such activity.

It is noteworthy that the biological activity disappears completely when the exocyclic group changes from -phenylaminide, 3, to -olate, 5. Further studies are proceeding in order to elucidate these relationships better.

Experimental

Melting points (uncorrected) were determined on a Kofler apparatus. Spectra were recorded on the following spectrometers: IR, Perkin Elmer, 710 A, in KBr pellets; ^{1}H NMR, Varian T-60, in DMSO-d_6 using TMS as internal reference; UV, Varian 634 S, 5 mg/L in acetonitrile or ethanol; Elemental analysis, Elemental microanalyser, Perkin Elmer 240. All the calculations were carried out using a MNDO-PM3 method (MOPAC 5.01) in a Cray XMP-28 at the University of London Computer Centre.

Aroyl chlorides were prepared by reacting the corresponding carboxylic acid with thionyl chloride. 1-Acyl-1-methylhydrazines were prepared by specific acylation on N-1 of 1-methylhydrazine at -50 °C. Phosgene was prepared by dropping carbon tetrachloride in fuming sulphuric acid at room temperature. 1-Acyl-1-alkyl-4-phenylthiosemicarbazides were prepared by the reaction of phenylisothiocyanate with the corresponding 1-acyl-1-alkylhydrazines (23).

General procedure for the preparation of 1,3,4-oxadiazolium-2-aminides, 3a-c

A mixture of an aroylthiosemicarbazide, 1, (4.1 mmoles) in acetone (15 ml) and yellow mercury (II) oxide (7.3 mmoles) was refluxed for 6 hours. The black precipitate of mercury (II) sulphide was removed by filtration and the filtrate was concentrated. The residual oil was dissolved in light petroleum ether from which it crystallized as a yellow solid. Yield: 70-75%. 3a, mp = 76-78 °C: Calculated (%) C = 58.89, H = 4.29, N = 17.17, found C = 58.98, H = 4.35, N = 16.99. γ 1630 cm^{-1}. λ_{max} = 240 nm, ϵ_{max} = 20,277, in acetonitrile. 3b, mp = 80 °C. 3c, mp = 75-76 °C: Calculated (%) C = 67.74, H = 4.30, N = 15.05, found C = 67.54, H = 4.13, N = 14.86.

For the preparation of 3d, the same procedure was followed, but using

1,4-diphenylthiosemicarbazide instead of an aroylthiosemicarbazide, 1. After separation of mercuric sulphide, acetylchloride was added to the filtrate, the mixture stirred for 30 min at room temperature and then left overnight. Acetone was removed under reduced pressure and the residual oil dissolved in ethanol, from which it crystallized as a yellow solid. Yield 87%, mp = 149 °C. δ 3.3 (3H), 6.9 (1H), 7.3-7.6 (10H). λ_{max} = 240 nm, ϵ_{max} = 15.612 in acetonitrile.

General procedure for the preparation of 1,3,4-oxadiazolium-2-olates, 5a, b

Freshly prepared phosgene (7.2 mmoles) was added to a mixture of 1-aroyl-1-methylhydrazine 4, (7.2 mmoles) and anhydrous potassium carbonate (1 g) in acetone (10 mL). Stirring continued with dry ice cooling for 1 hour. After standing overnight at room temperature, the mixture was refluxed for 1 hour, cooled to room temperature and filtered off. The filtrate was evaporated under reduced pressure leaving a residual yellow oil which crystallized after dissolving in toluene-petroleum ether. Average yield: 60%. 5a, mp = 172-174 °C: Calculated (%) C = 42.35, H = 2.35, N = 16.47; found: C = 41.95, H = 2.07, N = 15.97; δ 3.4 (3H), 7.6-8.0 (3H). λ_{max} = 249 nm, ϵ_{max} = 19,686, in acetonitrile. 5b. mp = 165 °C: Calculated (%) C = 47.81, H = 3.58, N = 16.82; found: C = 47.60, H = 3.02, N = 16.82. λ_{max} = 255 nm, ϵ_{max} = 16,566, in ethanol.

Microbiological assays

Preliminary assays with bacteria and fungi were carried out by the cupplate agar diffusion method,[24], using respectively Trypticase-soy agar (Difco) and Sabouraud-dextrose mixture and tested in concentrations of 500, 100, 50 and 10 μg/mL.

A two-fold serial dilution method in liquid medium (24) [Trypticase-soy broth or Sabouraud-dextrose broth (Difco)] was employed to determine the minimal inhibitory concentrations, MIC. Two sets of controls were used, the organisms control, without sample, and the solvent control. Antibiotic samples (streptomycin sulphate or nystatin), were also tested in the agar diffusion method.

Fungi studied were: *Aspergillus parasiticus*, *Microsporum canis*, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Cryptococcus neoformans*, *Rhodotorula rubra* (all from the Mycological collection of the Departamento de Microbiologia, Instituto de Ciências Biomédicas da Universidade de São Paulo).

Bacteria utilized were: *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853.

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References

(1) M. Ohta, and H. Kato, in J. P. Snyder *Non-Benzenoid Aromatics*, Academic Press, New York, 16, 117 1969

(2) W. D. Ollis, and C. A. Ramsden, *Adv. Heterocycl. Chem.* 19, 1, (1976)

(3) K. T. Potts, *Lect. Heterocycl. Chem.* 4, 35 (1978)

(4) (a) V. G. Yashunskii and V. V. Ogorodnikova, *Khimiya Geterotsiklicheskikh Soedinenii*, No. 3, 291 (1981) (b) V. A. Chuiguk, *ibidem*, No. 1, 3 (1983)

(5) C. G. Newton and C. A. Ramsden, *Tetrahedron*, 38(20), 2965, (1982)

(6) F. H. C. Stewart, *Chem. Rev.* 64, 129 (1964)

(7) V. G. Yashunskii and L. E. Kholodov, *Rus. Chem. Rev.* 40(1), 28, (1980)

(8) A. Echevarria and J. Miller, *J. Chem. Research (S)*, 391, (1987); *J. Chem. Research (M)*, 3187 (1987)

(9) T. O. Shinzato; N. F. Grynberg; R. M. Gomes; A. Echevarria and J. Miller, *Med. Sci. Res.* 17, 865 (1989)

(10) A. Echevarria and J. Miller, *J. Chem. Soc. Perkin Trans. II*, 1425 (1989)

(11) A. Echevarria and J. Miller. Kinetics of Reactions of 1,3-diphenyl-2-(4-chloro-3-nitrophenyl)-1,3,4-triazolium-2-thiolate with some anionic and neutral nucleophiles, *(IX IUPAC Conference on Physical Organic Chemistry)*, Regensburg, RFA, 1988, Abstract pp.98 (1988)

(12) R. C. S. C. Barbosa, A. Echevarria, A. M. Giesbrecht, J. Miller, C. A. Montanari, M. B. Oliveira and A. B. Pereira, Structure and Biological activity of some mesoionic compounds and precursors, *(7th European Symposium on Quantitative Structure-Activity Relationships)*, Interlaken, Switzerland (1988)

(13) M. B. Oliveira, J. Miller and A. B. Pereira, Synthesis of Mesoionic 2-alkylamino-1,3-dithiolium-4-thiolates, *3rd Brazilian Meeting on Organic Synthesis*, São Carlos, Brasil, Abstract PS. 4.09, 140 (1989)

(14) J. Miller, Y. Miyata and C. A. Montanari, On the synthesis of mesoionic 1,3,4-oxadiazolium-2-aminides, *(3rd Brazilian Meeting on Organic Synthesis)*, São Carlos, Brasil, Abstract PS 4.19, 150 (1989)

(15) R. M. Gomes, T. O. Shinzato, N. F. Grynberg, J. Miller and A. Echevarria, Inhibitory Effect of aryl-sydnones on mice tumour growth, *(15th International Cancer Congress)*, Hamburg, RFA, Abstract A.4.119.39, (1990) (Supplement of *Journal of Cancer Research and Clinical Oncology*, Vol. 116, (1990)

(16) (a) W. D. Ollis and C. A. Ramsden, *Chem. Commun.* 1223, (1971); (b) Ibidem, *J. Chem. Soc., Perkin Trans. I*, 638, 642 (1974)

(17) M. Alajarin and P. Molina, *Tetrahedron Letters*, 21, 4025 (1980)

(18) M. Hashimoto and M. Ohta, *Bull. Chem. Soc.* 34, 668 (1961)

(19) C. Ainsworth, *Can. J. Chem.* 43, 1607 (1965)

(20) J. J. P. Stewart, *J. Comput. Chem.* 10, 209 (1989)

(21) K. K. Cheung, A. Echevarria, S. Galembeck, M. A. M. Maciel, J. Miller, V. M. Rumjanek and A. M. Simas, *Acta Cryst.* C48, 1471 (1992)

(22) J. E. Smith, and M. O. Moss, *Mycotoxins. Formation, analysis and significance.* J. Wiley & Sons, N.Y. 1985

(23) C. A. Montanari, *M.Sc. Thesis*, Instituto de Química, Universidade de São Paulo, (1987)

(24) N. V. Lorian, *Antibiotics in Laboratory Medicine,* William & Wilkins Company, Baltimore, 1-192 (1980)

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