This experiment indicated that the observed peak was due to ethanol. A similar procedure was used to identify the first major peak. The peak resulted from the organic impurities in water.

RESULTS AND DISCUSSION

Table II shows a summary of the ethanol content of the three batches studied. The column material used in this investigation has outstanding separation properties for volatile, low-molecular weight compounds and was particularly valuable for this study since no signals were observed from any of the compounds in the dosage form except ethanol.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 28, 1969, from the *Analytical Laboratory, Astra Pharmaceutical Products, Inc., Worcester, MA 01606*

Accepted for publication February 17, 1970.

The author expresses his appreciation to William Webber and Dr. S. G. Davis for reviewing this manuscript.

Antimicrobial Activity of Some β-Nitrostyrenes

LEONARD R. WORTHEN and HOWARD W. BOND

Abstract □ Nine compounds were tested for antimicrobial activity, and eight of these exhibited varying spectra of inhibition.

Keyphrases □ β-Nitrostyrene derivatives—antimicrobial activity □ Antimicrobial activity, evaluation—β-nitrostyrene derivatives □ Paper disks—microbe-inhibition analysis

In the course of synthesizing some chemical compounds for testing as potential molluscicides, a number of β-nitrostyrene compounds were prepared. This report indicates the preliminary results obtained from testing nine of these compounds, seven of which are new (1), for antibacterial and antifungal activity.

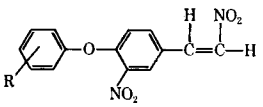
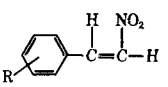
The antimicrobial activity of β-nitrostyrenes has been previously noted by several workers, including Schales and Graefe (2), Bocobo *et al.* (3), and Huitric *et al.* (4).

An impregnated filter paper disk on an inoculated agar plate was selected as the testing method. This technique has the advantage over the hole or cup method because the solvent can be evaporated from the saturated paper disks prior to placing them on the inoculated agar plate. In this manner, any potential antimicrobial effects from the solvent used can be avoided.

EXPERIMENTAL

Materials and Methods—The test organisms used included the following: *Staphylococcus aureus*, strain 209, ATCC 6538; *Escherichia coli*, ATCC 4157; *Pseudomonas aeruginosa*, ATCC 10145; *Bacillus subtilis; Proteus vulgaris; Aspergillus niger*, ATCC 9642; *Trichophyton mentagrophytes*, ATCC 9129; and *Candida albicans*, ATCC 10231. The melted test agar media were inoculated with 0.1 ml. of a 24-hr.-old bacterial culture grown in nutrient broth or a 0.1 ml. saline suspension of a 48-hr.-old fungal culture grown on Sabouraud's agar. Nutrient agar was employed for the bacterial tests

Table I—Antibacterial and Antifungal Activity of Some β-Nitrostyrenes

R	Microbial Spectrum[a] 1	2	3	4	5	6	7	8
								
H	++[b]	++	+	−	−	+	+	+++
p-CH_3O—	++	++	−	−	−	+	++	+++
p-CH_3CONH—	++	++	+	−	−	−	−	+
2,4-Cl_2—	++	+	−	−	−	−	+	+++
Cl_5—	+	−	−	−	−	−	−	−
Br_5—	−	−	−	−	−	−	−	−
								
m-CH_3CO_2—	++	++	++	++	++	+	++	++
p-$CH_3CH_2CO_2$—	++	++	+++	−	++	+	+++	+++
3-CH_3O-4-CH_3CO_2—	++	++	++	−	++	++	+++	+++

[a] Microbial spectrum: Gram-positive: 1, *S. aureus*, strain 209, ATCC 6538; 2, *B. subtilis*. Gram-negative: 3, *E. coli*, ATCC 4157; 4, *P. aeruginosa*, ATCC 10145; 5, *P. vulgaris*. Fungi: 6, *C. albicans*, ATCC 10231; 7, *A. niger*, ATCC 9642; *T. mentagrophytes*, ATCC 9129. [b] Zone of inhibition: − = less than 12.7 mm. (zone includes diameter of disk); + = less than 20 mm., ++ = 21–29 mm., and +++ = more than 30 mm.

and Sabouraud's agar for the fungal tests. All bacterial cultures were incubated at 35° and the fungal cultures at room temperature. The filter paper disks (12.7-mm. diameter) were saturated with 0.1 ml. of a 1:1000 concentration of the compounds to be tested; the solvent was allowed to evaporate before the disks were placed, in duplicate, on the seeded plates. Ethanolic solutions of eight of the compounds were used. The other compound [*m*-(2-nitrovinyl)-phenyl acetate] was employed as a suspension in pyridine. Disks impregnated with an equal amount of the solvent only were dried and used as controls and in no instance exhibited any inhibition. The zones of inhibition were read at the end of 24 hr. of incubation for the bacteria and 48 hr. for the fungi. The results are tabulated in Table I.

DISCUSSION

Nine β-nitrostyrene derivatives were tested by the paper disk method for antimicrobial activity. Of the six 3-nitro-4-substituted phenoxy-β-nitrostyrenes tested, the parent compound had the widest spectrum. The pentachloro derivative was effective against *S. aureus* only, and the pentabromo derivative was inactive. None of these compounds had any activity against *P. vulgaris* or *P. aeruginosa*.

The three ring-substituted β-nitrostyrenes tested all showed good activity against the test organisms. Of particular significance is their activity against the *Proteus* and *Pseudomonas* species tested. One limitation with the most active compound tested, *m*-acetoxy-β-nitrostyrene, was the inability to solubilize it in common organic solvents, even at a 1:1000 concentration. It was placed on the paper disk as a 1:1000 suspension. The results of the tests of the latter series of compounds warrant further study of other derivatives of this group.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 24, 1969, from the *Departments of Pharmaceutical Chemistry and Pharmacognosy, University of Rhode Island, Kingston, RI 02881*

Accepted for publication February 10, 1970.

This investigation was supported by the Office of Water Resources Research, U. S. Department of Interior, under P.L. 88-379.

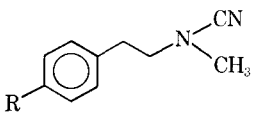
Solvent Effects in the NMR Spectra of Cyano Compounds

EDWARD E. SMISSMAN and ALEXANDROS C. MAKRIYANNIS

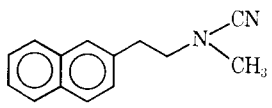
Abstract □ Novel solvent effects in the NMR spectra of certain cyano compounds are reported. A change in the splitting pattern of the methylene groups of *N*-cyano-*N*-methylarylethylamines with a change in solvent was observed in open-chain alkyl cyano compounds containing a terminal aromatic ring.

Keyphrases □ Solvent effects—NMR spectra, cyano compounds □ Cyano compounds, NMR—solvent effects □ NMR spectra—solvent effects, cyano compounds

During the synthesis of a number of *N*-cyano-*N*-methylarylethylamines (1), it was observed that the NMR spectra exhibited dramatic changes when the solvent was changed from either carbon tetrachloride or deuterochloroform to benzene. These changes involved: (*a*) a strong upfield shift in the singlet signal due to the *N*-methyl protons, and (*b*) a strong upfield shift of the multiplets due to the four methylene protons, accompanied by a pronounced change in the splitting pattern of the multiplet in all of the compounds where rotation was possible about the C—C bond of the ethylamine. The only compound examined which did not show the change in splitting pattern was *N*-cyanotetrahydroisoquinoline (Compound 5). The observations from the examined spectra are summarized in Table I.



1. R = H
2. R = OCH_3



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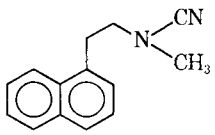

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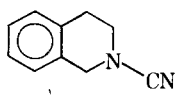

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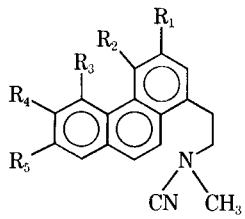
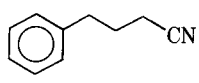


	R_1	R_2	R_3	R_4	R_5
6.	—OCH_2O—		OH	OMe	H
7.	—OCH_2O—		OAc	OMe	H
8.	H	H	OH	OH	H
9.	OH	OMe	H	OMe	OH



10

$ClCH_2CH_2CN$

11

$BrCH_2CH_2CH_2CN$

12

structures of compounds listed in Table I