NMR Spectroscopy and antibacterial activity of sulfur-substituted ß-hetarylacrylonitriles

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Dedicated to Professor Lubor Fisera on the occasion of his 60th birthday
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Abstract
A complete nmr spectroscopic characterization of a series of twenty four 5-thio- or 5-sulfonyl-substituted furyl-, thienyl-, and *N*-methylpyrrolylacrylonitriles was carried out. Where appropriate a three-bond long range C-H coupling constant was used for determining the stereochemistry of the double bond. Ten of the compounds were tested for antibacterial activity against three Gram-positive and three Gram-negative bacteria.

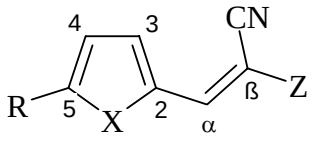
Keywords: Hetarylacrylonitriles, cinnamonitriles, sulfides, sulfones, nuclear magnetic resonance, antibacterial activity

Introduction

Within the framework of a project dealing with nucleophilic substitution reactions of hetaryl cinnamonitrile analogs a series of sulfinyl- and sulfonyl-substituted furyl-, thienyl-, and *N*-methylpyrrolylacrylonitriles was available.[1,5-10] This pool of compounds allowed us to study the interaction of the acrylonitrile moiety with two substituents of opposite electronic character through three different hetaryl rings by means of nmr spectroscopy.

Results and Discussion

Scheme 1 and Table 1 show an overview of all 24 compounds used for the nmr spectroscopic investigation.



R = MeS, $MeSO_2$, PhS, $PhSO_2$

X = O, S, NMe

Z = CN, COOMe

Scheme 1. Structure and numbering of compounds studied.

Table 1. Compounds studied

R		MeS	$MeSO_2$	PhS	$PhSO_2$
X	Z				
O	CN	**1**	**2**	**3**	**4**
S	CN	**5**	**6**	**7**	**8**
NMe	CN	**9**	**10**	**11**	**12**
O	COOMe	**13**	**14**	**15**	**16**
S	COOMe	**17**	**18**	**19**	**20**
NMe	COOMe	**21**	**22**	**23**	**24**

NMR spectroscopy

A full nmr spectroscopic characterization of all products including ^{1}H, ^{13}C, and ^{15}N (only for some of the pyrroles) spectra was performed.[2] Complete assignments of all signals based on 2D correlation experiments (HMQC and HMBC) and H-C coupling constants (*vide infra*) were carried out.

The structures of the investigated substances, with substituents being totally opposite in their electronic properties like thio and sulfonyl, permit to study how these functionalities affect the complete unsaturated system. The comparison of R-S- and R-SO_2- substituted, otherwise identical compounds clearly shows the transmission of the electronic effects through the heteroaromatic ring to the "acrylonitrile" group, in a way that parallels the molecules' possible mesomeric structures. By far the strongest influence is thus exerted on the carbon atom $C_ß$ with up to 12 ppm downfield shift, but effects on $C_α$ (up to 3 ppm downfield) and the carboxyl or nitrile atoms (1-2 ppm upfield) are also significant. The heterocycles differ in their effectiveness of transferring the mesomeric effect in the order pyrrole > furan > thiophene, possibly giving an indication of the relevance of non-aromatic resonance structures of the individual heterocyclic rings in the compounds studied.

Table 2. ^{1}H nmr data

Compd.	H_α	H_3	H_4	$J_{3,4}$	COOMe	NMe	R
1	8.06	7.46	6.81	3.9	-	-	2.67
2	8.46	7.53	7.55	3.9	-	-	3.41
3	7.35	7.32	6.51	3.8	-	-	7.55 – 7.40
4	8.38	7.48	7.67	3.8	-	-	8.05 - 7.70
5	8.46	7.79	7.21	4.1	-	-	2.71
6	8.83	7.97	7.99	4.1	-	-	3.49
7	8.50	7.82	7.29	4.0	-	-	7.55 – 7.40
8	8.77	7.91	8.01	4.1	-	-	8.10 – 7.60
9	8.11	7.51	6.57	4.5	-	3.66	2.57
10	8.50	7.38	7.03	4.7	-	3.99	3.39
11	8.30	7.50	6.71	4.5	-	3.74	7.40 – 7.15
12	8.40	7.38	7.20	4.5	-	3.83	8.0 – 7.70
13	7.95	7.50	6.73	3.8	3.80	-	2.65
14	8.27	7.58	7.53	3.8	3.86	-	3.41
15	7.93	7.42	6.60	3.8	3.90	-	7.35 – 7.50
16	8.19	7.53	7.63	3.8	3.83	-	8.10 – 7.70
17	8.46	7.95	7.23	4.0	3.82	-	2.70
18	8.72	8.12	7.98	4.1	3.88	-	3.47
19	8.49	7.98	7.39	4.1	3.81	-	7.50 – 7.40
20	8.66	8.06	8.02	4.2	3.85	-	8.10 – 7.60
21	8.03	7.58	6.56	4.7	3.79	3.70	2.54
22	8.22	7.42	7.02	4.5	3.86	4.00	3.38
23	8.15	7.57	6.76	4.4	3.83	3.74	7.40 – 7.10
24	8.14	7.42	7.18	4.7	3.83	3.83	8.0 – 7.60

Table 3. ^{13}C nmr shifts (part 1, R-hetaryl)

Compd.	C_2	C_3	C_4	C_5	NMe	R
1	148.7	128.8	112.5	160.9	-	14.4
2	150.2	123.8	118.2	153.6	-	42.4
3	149.8	125.2	116.7	158.2	-	133.4, 130.1, 129.8, 127.7
4	151.0	124.1	119.4	153.2	-	137.9, 135.2, 130.1, 127.9
5	133.2	142.1	126.0	156.2	-	17.9
6	140.1	139.2	133.5	149.5	-	44.8
7	136.0	141.2	130.8	150.4	-	132.5, 131.7, 129.9, 129.2
8	141.1	139.3	134.1	149.5	-	140.0, 134.6, 130.1, 127.4
9	128.8	119.6	113.1	142.3	31.2	15.8
10	131.4	115.5	117.3	136.7	32.4	44.4
11	130.0	118.1	119.8	133.9	31.4	132.5, 129.6, 128.3, 127.3
12	132.0	115.6	118.3	135.9	32.2	139.9, 134.4, 130.0, 127.4
13	148.8	127.6	112.1	158.8	-	14.5
14	150.7	123.0	118.0	152.9	-	42.3
15	150.8	123.4	117.8	154.9	-	132.3, 131.3, 129.9, 129.0
16	151.6	123.2	119.2	152.5	-	138.1, 134.9, 129.9, 127.8
17	133.7	142.0	126.3	153.6	-	18.0
18	140.7	139.1	133.3	148.4	-	44.9
19	137.3	141.2	132.2	146.8	-	133.5, 132.2, 130.9, 129.9
20	141.9	139.4	134.0	148.4	-	140.2, 134.5, 130.1, 127.3
21	128.4	118.9	113.0	139.9	30.1	16.4
22	131.4	114.8	117.0	135.8	32.0	44.5
23	130.0	117.4	119.9	134.7	31.1	129.8, 129.6, 127.5, 126.8
24	132.0	115.0	118.0	134.8	31.9	140.1, 134.2, 129.9, 127.2

Table 4. ^{13}C nmr shifts (part 2)

Compd.	C_α	$C_ß$	CN_{cis}	CN_{tr}	COOMe
1	141.7	71.2	114.0	115.1	-
2	144.5	81.0	112.5	113.6	-
3	141.6	76.2	113.0	114.4	-
4	144.1	81.2	112.4	113.6	-
5	151.4	72.4	114.1	114.7	-
6	152.7	81.2	112.9	113.5	-
7	151.8	75.1	113.6	114.3	-
8	152.4	81.9	112.8	113.4	-
9	143.5	65.2	115.8	116.3	-
10	146.6	77.5	113.8	114.4	-
11	145.6	70.5	114.8	115.3	-
12	146.6	77.9	113.7	114.3	-
13	137.2	93.8	115.8	-	163.1, 52.9
14	138.7	101.9	114.5	-	161.8, 53.5
15	138.8	97.8	115.6	-	163.4, 53.5
16	138.3	102.2	114.4	-	161.7, 53.4
17	146.5	94.8	116.2	-	162.9, 53.0
18	146.6	102.0	115.2	-	161.7, 53.5
19	146.7	97.4	115.8	-	162.5, 53.1
20	146.4	102.3	115.2	-	161.6, 53.5
21	139.0	89.0	117.5	-	164.0, 52.6
22	140.4	99.4	115.9	-	162.5, 53.3
23	140.3	93.5	116.6	-	163.2, 52.9
24	140.4	99.8	115.7	-	162.2, 53.2

Table 5. ^{15}N nmr shifts

Compd.	^{15}N
9	159.6
10	155.8
12	155.2
21	159.6
22	155.1

Finally fully ^{1}H-coupled ^{13}C nmr spectra were measured using the gated decoupling technique in order to record the complete proton-carbon coupling pattern. The one-bond H-C coupling constants again demonstrate the transmission of the electronic effects of the varying sulfur substituents through the unsaturated system: the value of ^{1}J at C_3, C_4, and C_α is

significantly higher (3 to 5 Hz) for R-SO_2- substituted compounds compared to their R-S- analogs, and electron withdrawing substitution is known to increase the coupling constants of the affected atoms.

Special attention was paid to the long range coupling between H_α and the CN or CO carbon atoms as a means of determining the stereochemistry of the double bond. The dihedral angle is the most important factor influencing the magnitude of a $^3J_{C,H}$, although there is some effect of the nature of the coupling carbon atom and the electronic properties of the substituents.[3] Therefore the fixed angles of ~0° and ~180° in olefinic structures make this coupling a useful tool in the differentiation between geometric isomers, as it has already been demonstrated, amongst others, for **1** and **13** and similar compounds.[4] Thus, for the cyanoacetates the *E* configuration could be proven for all compounds by a three bond coupling constant of the nitrile group in the range of 12.5 to 14 Hz (the *trans* relation to the proton results in a larger value) versus 6.5 to 7 Hz for the carboxyl C atom. In the same way for the malodinitriles the cyano group *cis* to the heterocyclic ring (coupling range again 12.5 to 14 Hz) could be distinguished from the *trans* nitrile (7.5 to 8.5 Hz). In both series these values also show a small increase of ~0.5 Hz for the sulfonyl substituted compounds.

Further long range couplings within and out of the heterocycles reflect the characteristic ranges of the individual rings and do not show any systematic influence of the varying substitution.

Table 6. One bond coupling constants

Compd.	C_3-H_3	C_4-H_4	C_α-H_α	Compd.	C_3-H_3	C_4-H_4	C_α-H_α
1	181.0	183.5	167.0	**13**	179.0	182.4	163.2
2	184.9	187.4	171.0	**14**	184.5	186.7	167.4
4	185.6	188.1	171.3	**16**	184.9	187.1	167.6
5	172.3	174.7	166.3	**17**	172.0	173.9	163.3
6	175.9	178.4	170.3	**18**	175.7	177.7	167.1
7	173.1	175.1	167.2	**19**	173.1	174.6	164.3
8	176.3	178.7	170.4	**20**	176.1	177.9	167.4
9	174.9	179.4	159.8	**21**	174.7	178.7	156.0
10	177.7	182.3	164.7	**22**	177.4	181.5	161.0
11	176.8	179.6	161.9	**23**	174.3	177.8	157.3
12	178.4	182.9	165.3	**24**	178.1	182.3	161.7

Table 7. Long range coupling constants (part 1, intra-heterocyclic)

Compd.	C_2-H_3	C_2-H_4	C_3-H_4	C_4-H_3	C_5-H_3	C_5-H_4
1	9.8	8.2	-[a]	4.2	9.4	9.4
2	10.0	8.3	3.1	3.6	8.6	8.6
4	9.9	8.4	2.9	3.6	8.7	8.7
5	6.3	11.1	4.8	5.3	12.7	5.4
6	6.9	11.0	4.5	4.9	11.0	5.3
7	6.0	11.2	4.9	5.1	12.9	5.2
8	6.6	11.3	4.5	5.0	11.4	5.0
9	8.6	8.6	3.7	3.4	-[a]	-[a]
10	8.8	8.8	3.6	3.1	8.8	6.2
11	8.5	8.5	4.1	2.9	9.3	9.3
12	8.8	8.8	3.1	2.7	8.9	6.0
13	9.6	8.1	-[a]	4.3	9.2	9.2
14	9.8	8.2	3.5	4.1	8.6	8.6
16	9.7	8.3	3.2	4.1	8.5	8.5
17	6.2	11.0	4.9	5.5	12.7	5.2
18	6.4	11.2	4.5	5.2	11.4	5.2
19	5.9	11.0	5.0	5.3	13.1	5.0
20	6.5	11.2	4.4	5.3	11.4	5.0
21	8.4	8.4	3.8	3.6	-[a]	-[a]
22	8.7	8.7	3.6	3.5	8.5	6.0
23	8.3	8.3	4.4	2.8	8.0	8.0
24	8.7	8.7	3.1	2.9	-[a]	-[a]

[a] Not resolved

Table 8. Long range coupling constants (part 2)

Compd.	C_2-H_α	C_3-H_α	C_α-H_3	$C_ß$- H_α	CN_{cis}-H_α	CN_{tr}-H_α	CO-H_α
1	4.5	-[a]	-[a]	1.6	13.1	7.8	-
2	3.4	2.4	1.2	2.1	13.7	8.2	-
4	4.0	2.3	1.3	2.2	13.9	8.3	-
5	2.6	4.8	3.9	0.7	13.1	7.8	-
6	2.2	4.5	3.7	1.2	13.7	8.2	-
7	2.4	4.9	4.0	0.7	13.4	8.0	-
8	2.2	4.5	3.7	1.3	13.7	8.3	-
9	3.0	5.6	0.8	2.1	12.8	7.8	-
10	2.8	5.2	0.8	2.5	13.5	8.2	-
11	2.8	5.2	-[a]	-[a]	13.2	8.1	-
12	2.9	5.2	-[a]	2.1	13.5	8.4	-
13	5.0	-[a]	1.3	2.3	13.0	-	6.4
14	4.1	2.4	1.3	2.5	13.5	-	6.6
16	4.6	2.4	1.4	2.5	13.7	-	6.7
17	3.6	4.9	4.1	1.2	12.9	-	6.4
18	3.2	4.5	3.8	-[a]	13.4	-	6.6
19	3.4	5.0	4.0	1.1	13.1	-	6.4
20	3.2	4.4	3.6	1.5	13.5	-	6.6
21	2.8	5.7	0.9	2.5	12.6	-	6.3
22	2.8	5.3	-[a]	3.1	13.2	-	6.6
23	2.8	5.1	-[a]	-[a]	12.8	-	6.5
24	2.9	5.0	-[a]	-[a]	13.2	-	7.1

[a] Not resolved

Testing for antibacterial activity

The *in vitro* antibacterial data (MIC: minimum inhibitory concentration) of selected compounds against three Gram-negative bacteria (*Escherichia coli* 326/71, *Pseudomonas aeruginosa* 133/71, and *Serratia marcescens* 303) and three Gram-positive bacteria (*Staphylococcus aureus* 78/71, *Enterococcus faecalis* 1875, and *Bacillus subtilis* 2216) compared to data for nalidixic acid. None of the compounds showed a minimum inhibitory concentration of the level of nalidixic acid against Gram-negative bacteria. Two compounds (**2** and **14**) showed twice better activity than nalidixic acid against *E. faecalis*. Compound **13** exhibited the highest effect against *E. faecalis*, its MIC was 64 µg/ml. *E. faecalis* was the most susceptible organism. Generally, the tested compounds have no intrinsic antibacterial activity against selected microorganisms.

Experimental Section

Synthesis of compounds. Most of the compounds used within this study were synthesized according to known procedures, and physical properties were in agreement with the values given there: **1**, **2**, **13**, and **14**;[5] **3** and **15**;[6] **4**;[7] **5**, **7**, **8**, **19**, and **20**;[8] **9-12** and **21-24**;[9] and **16**.[10]

5-Methylsulfonyl-2-thienylmethylenemalodinitrile (**6**) was prepared from **5** by oxidation with hydrogen peroxide in acetic acid according to a procedure given for **2**.[5]

Methyl 5-methylthio-2-thienylmethylenecyanoacetate (**17**) was prepared by condensation of 5-methylthio-2-thiophencarbaldehyde and methyl cyanoacetate analogous to **5**.[8]

Methyl 5-methylsulfonyl-2-thienylmethylenecyanoacetate (**18**) was prepared from **17** by oxidation with hydrogen peroxide in acetic acid according to a procedure given for **14**.[5]

Complete nmr data of **6**, **17**, and **18** can be found in the tables in the **Results** section. Elementary analyses agreed within +/- 0.3% with the calculated values.

NMR spectroscopy. Nmr spectra were recorded on a Bruker Avance DRX 400 spectrometer controlled by a Silicon Graphics O2 workstation. Using a 5 mm inverse broadband probehead, spectra were acquired from solutions of approximately 20-40 mg/mL in DMSO-d_6 at 300 K and referenced to TMS.

^{1}H nmr spectra (400.13 MHz) were recorded using 30° pulse angle, 4.8 kHz spectral width, pulse repetition time 4.5 s, 32k data points, and 16 scans. ^{1}H-^{13}C HMQC and HMBC spectra were recorded with 2k data points and 16-32 scans for each FID and 256 increments.

Instrumental settings for CPD decoupled ^{13}C nmr spectra (100.62 MHz) were: 30° pulse angle, 25.1 kHz spectral width, pulse repetition time 2.3 s, 64k data points, up to 8000 scans. Exponential multiplication (1 Hz) was applied. For gated decoupled ^{13}C nmr spectra 128k data points were recorded with a pulse repetition time of 3.6 s, followed by zero-filling to 256k. No EM was used and 8000 to 12000 scans were necessary.

^{15}N nmr spectra (40.56 MHz) were obtained from solutions of approximately 40 mg/mL in DMSO-d_6 at 300 K using the INEPT method with delays calculated for J = 10 Hz and a pulse repetition rate of 2.7 s. 20.3 kHz spectral width and 32 k data points were used for all spectra. Up to 8000 scans were necessary depending on concentration. Exponential multiplication (1 Hz) was applied. Shifts are given relative to liquid ammonia as secondary reference at 380.2 ppm high-field from nitromethane as primary reference.[11]

Biological testing. The microorganisms used in the study were obtained from the Czech National Collection of Type Cultures, Prague (*Escherichia coli* 326/71, *Pseudomonas aeruginosa* 133/71, *Staphylococcus aureus* 78/71) and from the Czech Collection of Microorganisms, Brno (*Serratia marcescens* 303, *Enterococcus faecalis* 1875, *Bacillus subtilis* 2216). Nalidixic acid was obtained from Sigma (St. Louis, Mo.). Broth macrodilution susceptibility tests were performed by standard methods. Serial twofold dilutions of compound solutions were prepared in Mueller-Hinton broth supplemented with 25 mg of magnesium and 50 mg of calcium per ml. A standard inoculum (5 x 105 CFU) was added to each tube. All tubes were incubated for 18 h at 37˚C and then examined for turbidity by comparison with the growth

control tube (containing no tested compound). The MIC was defined as the lowest concentration of compound which inhibited visible growth.

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