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Reversed-phase high-performance liquid chromatographic study of the lipophilicity of a series of analogues of the antibiotic "calvatic acid"

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ABSTRACT

The lipophilicity of a series of *para*- and *meta*-substituted phenyl-ONN-azoxycyanides, analogues of the antibiotic 'calvatic acid', was studied by reversed-phase high-performance liquid chromatography using methanol–water or acetonitrile–water as the mobile phase and a LiChrospher 100 RP-18 column. An excellent linear relationship between the logarithm of the capacity factors (log k') for each compound and the volume fraction of the organic modifier (φ) was found. The extrapolation of log k' to $\varphi = 0$ gives log k_w for every compound. From these values τ_w constants were calculated for each substituent. Good correlations were found between log k_w and log P_{oct/H_2O} and between τ_w and Hansch's π hydrophobic parameter.

INTRODUCTION

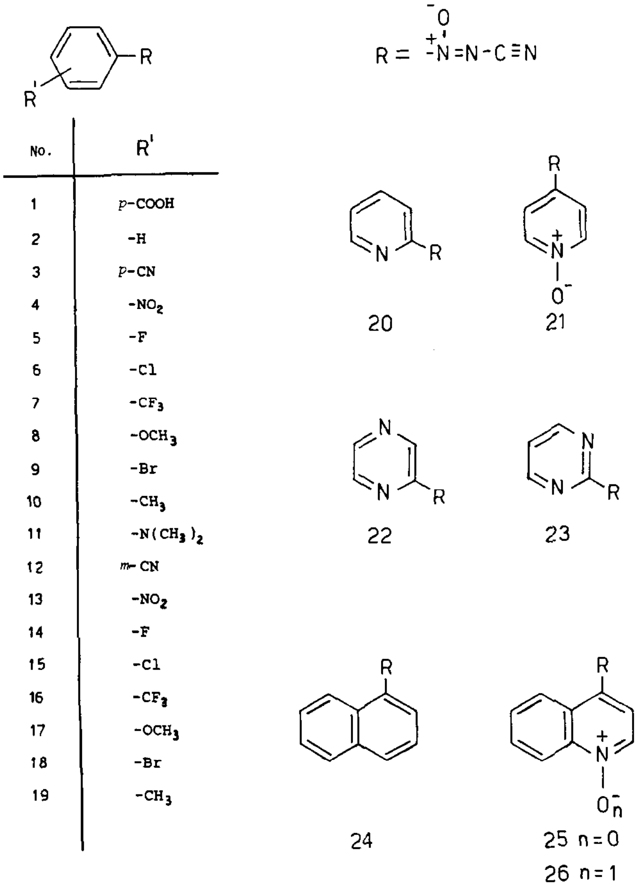
The cyano-NNO-azoxy group (ONN-azoxycyanide group) is an interesting function present in "calvatic acid" (**1**), an antibiotic produced by some Basidiomycetes [1,2]. This moiety is responsible for the antimicrobial properties of the antibiotic [3,4].

The ability of **1** to inhibit [^{3}H]colchicine binding to rat liver soluble tubulin [2] prompted us to prepare and test for their potential antitumour properties several new derivatives bearing the cyano-NNO-azoxy group linked to different vectors [5].

In recent work we showed that the electronic and hydrophobic constants of this function are similar to those of the nitro group [6]. In this paper, in order to complete the physico-chemical characterization of the –N(O)=NCN group, we report the results of an investigation in which the aryl-ONN-azoxycyanides **2**–**19** and **24** and the heteroaryl-ONN-azoxycyanides **20**–**23**, **25** and **26** were studied by reversed-phase high-performance liquid chromatography (RP-HPLC).

EXPERIMENTAL

Azoxycyanide derivatives **2**–**26** (Fig. 1) were synthesized and purified according to methods reported previously [5,6]. Methanol and acetonitrile were of analytical-reagent grade. The partition coefficients of the derivatives **20**–**26** were obtained using the shake-flask method at room temperature using octanol as lipid phase and water as



No.	R'
1	p-COOH
2	-H
3	p-CN
4	$-NO_2$
5	-F
6	-Cl
7	$-CF_3$
8	$-OCH_3$
9	-Br
10	$-CH_3$
11	$-N(CH_3)_2$
12	m-CN
13	$-NO_2$
14	-F
15	-Cl
16	$-CF_3$
17	$-OCH_3$
18	-Br
19	$-CH_3$

Fig. 1. Formulae of compounds **1**–**26**.

hydrophilic phase. Each tabulated log *P* value is the average of four determinations made with different concentrations of solute (10^{-3}–10^{-4} *M*) (Tables I and II). No concentration dependence of the partition coefficients was observed. Owing to the low pK_a of compound **11**, no correction for partial ionization was taken into account.

Linear regression analyses were performed by the usual procedure on a IBM AT personal computer. Standard errors of the slope and intercept are reported in parentheses.

Chromatographic procedure

RP-HPLC experiments were performed on a Perkin-Elmer Series 2B liquid chromatograph equipped with a Perkin-Elmer LC75 variable-wavelength detector and a Rheodyne Model 7105 injection valve. A Perkin-Elmer LCI 100 integrator was used. A LiChrospher 100 RP-18 (10 μm) column (250 × 4.0 mm I.D.) (Merck) was employed.

Methanol–water or acetonitrile–water mixtures at various compositions (φ) were used as mobile phases.

The samples were dissolved in acetonitrile at concentrations suitable for UV detection and 1–5 μl of solution were injected into the chromatographic column. Each chromatographic run was repeated at least three times. Measurements of the retention times (t_R) were taken at room temperature.

The flow-rate was 1.5 ml/min and the column dead time (t_0) was determined according to the Knox and Kaliszan method [7]. From the solute retention time t_R, the logarithm of the capacity factor, log k', was calculated by the equation

$$\log k' = \log\left(\frac{t_R - t_0}{t_0}\right) \qquad (1)$$

RESULTS AND DISCUSSION

Retention parameters

Generally, the dependence of log k' on mobile phase composition is complex [8]. By using methanol–water mixtures as mobile phases and working with non-ionized solutes, the linear relationship between log k' and the volume fraction of organic modifier

$$\log k' = \log k_w - a\varphi \qquad (2)$$

where k_w is the capacity factor for pure water as a mobile phase and a is a constant, holds over the range $10 \leqslant \varphi \leqslant 80$ [9].

Log k' values for *meta-* and *para*-substituted phenyl-ONN-azoxycyanide and heteroaryl-ONN-azoxycyanide derivatives **2–26** measured at various methanol–water ratios (φ_M) are reported in Table I. The values in Table I show for each compound an excellent linear relationship with the φ_M values (see Table III) over the whole volume fraction range considered according to eqn. 2. From these correlations it was possible to extrapolate for each derivative the corresponding log $k_{w(M)}$ value.

Similar results were obtained using acetonitrile–water mixtures ($20 \leqslant \varphi \leqslant 80$) as the mobile phase (see Tables II and III). As an example, Fig. 2 shows the plot of log k' *vs.* volume fraction for both methanol and acetonitrile for compound **5**. For this series of derivatives, the log $k_{w(M)}$ values are always greater than the corresponding log $k_{w(A)}$ values. Log $k_{w(M)}$ is correlated with log $k_{w(A)}$ according to the equation

$$\log k_{w(M)} = -0.16(\pm 0.11) + 1.28(\pm 0.05)\log k_{w(A)} \qquad (3)$$

$$n = 25;\ s = 0.160;\ r = 0.980$$

Relationship between capacity factors and partition coefficients

Log k' values at various methanol–water and acetonitrile–water compositions correlate very well with log P values measured in the reference system n-octanol–water. The correlation coefficient r and the standard deviation s were spread over the ranges r = 0.969–0.996, s = 0.234–0.080 for the methanol–water system and r = 0.962–0.988, s = 0.227–0.112 for the acetonitrile–water system.

Log $k_{w(M)}$ and log $k_{w(A)}$ are strongly correlated to log P by means of the

TABLE I

LOG k' VALUES WITH METHANOL–WATER MOBILE PHASES

Compound	Volume fraction of organic modifier, $\varphi_{(M)}$							
	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80
2		1.74	1.44	1.13	0.75	0.42	0.13	−0.22
3	1.78	1.33	0.98	0.65	0.29	−0.04	−0.32	−0.71
4		1.44	1.11	0.82	0.46	0.13	−0.15	−0.52
5		1.66	1.35	1.05	0.69	0.34	0.05	−0.33
6			1.74	1.40	0.97	0.60	0.29	−0.10
7			1.86	1.49	1.06	0.68	0.24	−0.18
8			1.74	1.40	0.97	0.57	0.26	−0.11
9			1.86	1.50	1.08	0.67	0.35	−0.06
10			1.87	1.52	1.08	0.68	0.36	−0.04
11				1.68	1.11	0.69	0.37	−0.02
12	1.86	1.36	1.00	0.65	0.33	0.01	−0.27	−0.62
13		1.52	1.19	0.91	0.56	0.23	−0.07	−0.42
14		1.74	1.42	1.14	0.78	0.43	0.13	−0.23
15			1.79	1.48	1.06	0.69	0.35	−0.02
16			1.89	1.54	1.10	0.67	0.30	−0.13
17			1.75	1.43	1.00	0.61	0.28	−0.07
18			1.92	1.58	1.15	0.75	0.40	0.02
19			1.87	1.53	1.09	0.69	0.36	−0.02
20	1.30	0.89	0.57	0.29	0.02	−0.22	−0.44	−0.70
21	0.58	0.19	−0.11	−0.36	−0.66	−0.77	−1.01	−1.44
22	0.76	0.42	0.16	−0.06	−0.27	−0.44	−0.66	−0.94
23	0.63	0.31	0.06	−0.16	−0.35	−0.52	−0.71	−0.98
24				1.83	1.33	0.86	0.48	0.06
25			1.66	1.31	0.82	0.43	0.11	−0.24
26		1.52	1.08	0.77	0.34	0.02	−0.24	−0.55

Collander-type equations

$$\log P = -1.07(\pm 0.10) + 1.16(\pm 0.04)\log k_{w(M)} \tag{4}$$

$$n = 25;\ s = 0.146;\ r = 0.988$$

and

$$\log P = -2.06(\pm 0.33) + 1.82(\pm 0.14)\log k_{w(A)} \tag{5}$$

$$n = 25;\ s = 0.150;\ r = 0.952$$

Group contribution to hydrophobicity

From the log k_w values reported in Table III, it is possible to calculate the hydrophobic constants τ_w [10] (see Table IV) according to the equation

$$\tau_w = \log(k_{wj}/k_w) \tag{6}$$

TABLE II

LOG k' VALUES WITH ACETONITRILE–WATER MOBILE PHASES

Compound	Volume fraction of organic modifier, $\varphi_{(A)}$							
	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80
2		1.47	1.07	0.72	0.39	0.10	−0.16	−0.42
3	1.63	1.23	0.88	0.56	0.24	−0.07	−0.34	−0.67
4		1.40	1.03	0.69	0.35	0.03	−0.26	−0.59
5		1.45	1.08	0.73	0.40	0.10	−0.17	−0.46
6		1.83	1.39	0.97	0.61	0.28	0.01	−0.28
7			1.58	1.11	0.68	0.32	0.01	−0.31
8		1.75	1.25	0.83	0.47	0.16	−0.10	−0.38
9			1.48	1.06	0.66	0.33	0.05	−0.23
10		1.89	1.39	0.98	0.59	0.28	0.01	−0.26
11		1.91	1.34	0.87	0.50	0.20	−0.06	−0.32
12	1.59	1.23	0.85	0.52	0.22	−0.09	−0.37	−0.66
13		1.44	1.02	0.66	0.34	0.02	−0.27	−0.57
14		1.54	1.12	0.75	0.43	0.12	−0.15	−0.42
15		1.90	1.41	0.98	0.62	0.29	0.02	−0.24
16			1.55	1.07	0.67	0.31		−0.30
17		1.80	1.28	0.87	0.52	0.20	−0.07	−0.34
18			1.50	1.04	0.67	0.34	0.06	−0.20
19		1.90	1.40	0.97	0.61	0.29	0.02	−0.24
20	0.95	0.61	0.33	0.10	−0.13	−0.36	−0.59	−0.81
21	0.41	0.06	−0.24	−0.48	−0.72	−0.98	−1.26	−1.43
22	0.62	0.37	0.15	−0.05	−0.26	−0.47	−0.71	−0.96
23	0.49	0.24	0.02	−0.17	−0.37	−0.58	−0.83	−1.07
24			1.72	1.19	0.76	0.41	0.11	−0.16
25		1.61	1.09	0.68	0.35	0.08	−0.17	−0.39
26	1.64	1.07	0.63	0.27	−0.01	−0.25	−0.50	−0.69

where k_w is the capacity factor for 100% water eluent of the parent azoxycyanide **2** and k_{wj} is that of substituted compound j.

As the methanol–water mobile phase is the best HPLC reference system for determining hydrophobic parameters [11], we investigated correlations between $\tau_{w(M)}$ and $\pi_{N(O)=NCN}$ derived from the phenylazoxycyanide system [6] according to the equation

$$\tau_{w(M)} = 0.13(\pm 0.03) + 0.85(\pm 0.06)\pi \tag{7}$$

$$n = 18;\ s = 0.124;\ r = 0.958$$

No improvement was obtained by treating *meta*- and *para*-substituted derivatives as separate series. Similar results ($r = 0.950, s = 0.079$) were obtained for the correlation between $\tau_{w(A)}$ and π values.

In a previous study [6] we found that the $\pi_{RC_6H_5-N(O)=NCN}$ scale derived from

TABLE III

SLOPES AND LOG k_w VALVES IN EQN. 2 FOR AZOXYCYANIDES AND LOG P VALUES

Compound	Log P	Methanol			Acetonitrile		
		Log k_w	Slope	r	Log k_w	Slope	r
2	1.87	2.41 (±0.02)	−3.29 (±0.05)	0.999	2.01 (±0.06)	−3.12 (±0.11)	0.997
3	1.18	2.05 (±0.03)	−3.47 (±0.07)	0.999	1.88 (±0.03)	−3.22 (±0.06)	0.999
4	1.51	2.10 (±0.02)	−3.26 (±0.04)	0.999	2.03 (±0.03)	−3.30 (±0.05)	0.999
5	1.80	2.34 (±0.02)	−3.30 (±0.04)	0.999	2.03 (±0.04)	−3.17 (±0.08)	0.998
6	2.33	2.85 (±0.04)	−3.70 (±0.07)	0.999	2.44 (±0.08)	−3.50 (±0.15)	0.996
7	2.47	3.10 (±0.03)	−4.10 (±0.05)	0.999	2.62 (±0.09)	−3.75 (±0.16)	0.996
8	2.15	2.86 (±0.04)	−3.74 (±0.08)	0.999	2.31 (±0.10)	−3.48 (±0.19)	0.993
9	2.50	3.02 (±0.04)	−3.86 (±0.06)	0.999	2.42 (±0.09)	−3.40 (±0.16)	0.999
10	2.39	3.03 (±0.04)	−3.85 (±0.07)	0.999	2.47 (±0.10)	−3.56 (±0.18)	0.993
11	2.30	3.24 (±0.16)	−4.14 (±0.26)	0.994	2.45 (±0.14)	−3.63 (±0.26)	0.987
12	1.15	2.08 (±0.05)	−3.43 (±0.11)	0.997	1.85 (±0.04)	−3.19 (±0.07)	0.998
13	1.45	2.15 (±0.02)	−3.21 (±0.03)	0.999	2.03 (±0.05)	−3.30 (±0.09)	0.998
14	1.85	2.42 (±0.03)	−3.28 (±0.05)	0.999	2.10 (±0.06)	−3.23 (±0.12)	0.997
15	2.40	2.91 (±0.04)	−3.67 (±0.06)	0.999	2.48 (±0.10)	−3.53 (±0.18)	0.993
16	2.52	3.14 (±0.04)	−4.07 (±0.07)	0.999	2.57 (±0.10)	−3.68 (±0.17)	0.996
17	2.24	2.86 (±0.04)	−3.69 (±0.07)	0.999	2.36 (±0.10)	−3.49 (±0.19)	0.992
18	2.56	3.08 (±0.03)	−3.83 (±0.06)	0.999	2.42 (±0.10)	−3.37 (±0.18)	0.995
19	2.49	3.02 (±0.04)	−3.81 (±0.07)	0.999	2.47 (±0.10)	−3.54 (±0.20)	0.992
20	0.53	1.45 (±0.06)	−2.77 (±0.11)	0.995	1.12 (±0.04)	−2.46 (±0.07)	0.997
21	−0.41	0.75 (±0.06)	−2.66 (±0.13)	0.993	0.60 (±0.04)	−2.62 (±0.07)	0.997
22	0.10	0.90 (±0.04)	−2.30 (±0.08)	0.996	0.83 (±0.01)	−2.20 (±0.03)	0.999
23	−0.13	0.77 (±0.04)	−2.18 (±0.08)	0.996	0.70 (±0.02)	−2.18 (±0.04)	0.998
24	3.17	3.54 (±0.08)	−4.38 (±0.13)	0.999	2.71 (±0.14)	−3.71 (±0.24)	0.992
25	2.07	2.80 (±0.08)	−3.86 (±0.13)	0.998	2.10 (±0.12)	−3.26 (±0.23)	0.988
26	1.12	2.14 (±0.06)	−3.44 (±0.12)	0.997	1.72 (±0.12)	−3.23 (±0.24)	0.984

substituted phenyl-ONN-azoxycyanides and the $\pi_{RC_6H_5NO_2}$ scale derived from substituted nitrobenzenes [12] are strongly correlated by the equation

$$\pi_{RC_6H_5N(O)=NCN} = -0.02(\pm 0.02) + 0.96(\pm 0.03)\pi_{RC_6H_5NO_2} \tag{8}$$

$$n = 18;\ s = 0.06;\ r = 0.991$$

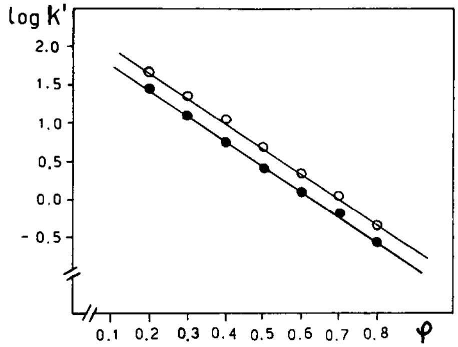


Fig. 2. Log k' *versus* volume fraction (○ = φ_M; ● = φ_A) for compound **5**.

TABLE IV

HYDROPHOBIC SUBSTITUENT CONSTANTS, τ_w, DERIVED FROM TABLE III

Compound	Methanol–water: $\tau_{w(M)}$	Acetonitrile–water: $\tau_{w(A)}$	π
2	0	0	0
3	−0.36	−0.13	−0.69
4	−0.31	0.02	−0.36
5	−0.07	0.02	−0.07
6	0.44	0.43	0.46
7	0.69	0.61	0.60
8	0.45	0.30	0.28
9	0.61	0.41	0.63
10	0.62	0.46	0.52
11	0.83	0.44	0.43
12	−0.33	−0.16	−0.72
13	−0.26	0.02	−0.42
14	−0.01	0.09	−0.02
15	0.50	0.47	0.52
16	0.73	0.56	0.65
17	0.45	0.35	0.37
18	0.67	0.41	0.69
19	0.61	0.46	0.62

This means that it is possible to derive, from the equations discussed above, retention parameters for azoxycyanide derivatives from lipophilicity parameters for the corresponding nitrobenzenes.

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