DC POLAROGRAPHIC AND UV SPECTROMETRIC STUDIES OF SUBSTITUTED FURO[3,2-*b*]- AND FURO[2,3-*b*]PYRROLES

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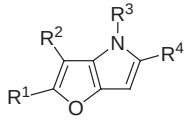
The aim of the synthesis of substituted furo[3,2-*b*]- and furo[2,3-*b*]pyrroles was to discover biologically active compounds. On that account, their potential carcinogenicity was tested by DC polarographic assay in the presence of α-lipoic acid. Only one of the studied compounds showed a marginal carcinogenic activity, the other exhibited only a negligible carcinogenic potential under the conditions studied. Their apparent ionization constants, determined by spectrometric titration, were correlated with their structure. The results confirm that the presence of methyl, furyl and/or phenyl groups affects the ionization.

Keywords: Fused heterocycles; Furo[3,2-*b*]pyrroles; Furo[2,3-*b*]pyrroles; Furans; Pyrroles; DC polarography; Electroreductions; UV spectroscopy; Ionization constants; Carcinogens.

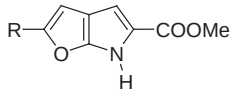
Efficient synthetic methods for indole (furopyrroles, thienopyrroles) and quinoline isosters (furopyridines, thienopyridines) in which the benzene ring is replaced with furan or thiophene rings are of great interest[1]. Investigations of these types of heterocycles have resulted in discovering many biologically active compounds[2]. Recently syntheses, as well as addition and cycloaddition reactions, of substituted furo[2,3-*b*]pyrroles[3–6] have been reported. A comparative study of Diels–Alder reactions of furo[2,3-*b*]pyrroles and their isomeric furo[3,2-*b*]pyrroles has revealed that the former system is a more active diene than the latter[4]. However, the reactivity of both 2-formyl derivatives is comparable[5]. *Ab initio* calculations were used to interpret different behavior of furo[2,3-*b*]pyrroles and furo[3,2-*b*]pyrroles[6].

Syntheses and properties of compounds **1a**–**1i** are described in detail in refs[7–10]. By reactions of furan-type aldehydes [furan-2-carbaldehyde, 5-methylfuran-2-carbaldehyde, 4,5-dimethylfuran-2-carbaldehyde, 5-phenylfuran-2-carbaldehyde and 5-(4-chlorophenyl)furan-2-carbaldehyde] with

methyl azidoacetate in the presence of sodium methoxide the corresponding azidoacrylates were obtained. These compounds gave by thermolysis in boiling toluene the corresponding methyl furo[3,2-*b*]pyrrole-5-carboxylates **1a**–**1d**. *N*-Methyl derivative **1e** was prepared under conditions of phase-transfer catalysis, while the 2-formylated product **1f** was prepared from **1a** using the Vilsmeier reaction. Acids **1g**, **1h** and **1i** were prepared by alkaline hydrolysis of esters **1a**, **1b** and **1c**, respectively, and subsequent acidification with hydrochloric acid at low temperature. Compound **1f** gave with hydroxylamine hydrochloride and acetic anhydride in the presence of pyridine the 2-cyano derivative[10], which was hydrolysed under standard conditions to give 4*H*-furo[3,2-*b*]pyrrole-2,5-dicarboxylic acid (**1j**). Methyl furo[2,3-*b*]pyrrole-5-carboxylate (**2a**) was prepared[3] from furan-3-carbaldehyde analogously to its isomer **1a**. Formylation of **2a** under the Vilsmeier conditions occurred[3] at C2 and led to **2b**.



1	R^1	R^2	R^3	R^4
a	H	H	H	CO_2Me
b	CH_3	H	H	CO_2Me
c	CH_3	CH_3	H	CO_2Me
d	C_6H_5	H	H	CO_2Me
e	4-Cl-C_6H_4	H	CH_3	CO_2Me
f	CHO	H	H	CO_2Me
g	H	H	H	CO_2H
h	CH_3	H	H	CO_2H
i	CH_3	CH_3	H	CO_2H
j	CO_2H	H	H	CO_2H



2	R
a	H
b	CHO

Polarographic behavior of compounds **1a**–**1j**, **2a** and **2b** in aprotic solvents and their biological activity have not been described yet. The aim of the preparation of these newly synthesized compounds was their application as potential cytostatics. It is known that many antineoplastics are also efficient carcinogens[11]. As a consequence of the fact, the secondary-

transformed tumors are induced after successful chemotherapy with some antineoplastics (alkylating agents, hormones). Carcinogenic activity of chemical compounds depends on their physical properties, structure and the number of rings in molecule (*e.g.* molecules of carcinogenic polyaromatic hydrocarbons contain 4–6 rings). On the other hand, carcinogenicity of substances can also be affected by their substitution. Aza analogues of polyaromatic hydrocarbons possess lower carcinogenic activity than their parent compounds. The aim of organic chemists is to synthesize such antineoplastics that save healthy cells and decrease the number of tumor cells (*e.g. via* apoptosis). In the past decade, the testing of carcinogenicity has been performed using mostly experimental animals, whereas nowdays, they are partially replaced by the series of mutagenic tests (Ames test, chromatid exchange test *etc*.). All of these tests are elaborate and time-consuming. From the reasons described above, the electrochemical test for carcinogenic activity with α-lipoic acid (D,L-6,8-tioctic acid) has been developed[12]. This electrochemical test using DC polarography is simple, rapid and financially bearable. Determination of potential carcinogenicity by DC polarography is based on the observation of their polarographic reduction course in the presence of α-lipoic acid, which is a well-known modulator of carcinogenic processes. α-Lipoic acid, a growth factor for many bacteria and protozoa, is dominant part of hepatal S9 mix fraction that activates mutagens in the Ames test on *Salmonella typhimurium*. The method for determination of potential carcinogenic activity has been described in detail earlier[12–16]. Polarographic behavior of α-lipoic acid in strictly anhydrous DMF showed, that at concentrations up to 10^{-2} mol dm^{-3} exhibits no polarographic wave of its own but affects the polarographic reduction of carcinogenic compounds[12]. During the polarographic reduction of such compounds in the presence of α-lipoic acid, the value of the diffusion current i_d (μA) of the first polarographic wave increases[12,13] or appears a new polarographic wave[17,18]. The increase of current i_d with increasing concentration of α-lipoic acid is linear and follows the linear equation of $y = kx + q$, where y means diffusion current, i_d, increases of the first original or new polarographic wave, x represents the concentration of α-lipoic acid and k and q are constants. The tangent of angle α between the straight line and x coordinate is used as a basis for evaluating the potential carcinogenic activity of the tested compounds. The values of angle α for some groups of chemical compounds with known carcinogenic activity were also published[12,18] and are in strong relation to the level of their carcinogenicity. This carcinogenic activity is expressed as a parameter of potential carcinogenic activity tg α. Compounds with tg α lower than 0.100 are considered to be non-carcinogenic,

values of tg α in the range from 0.101 to 0.200 are indicative of a marginal carcinogenicity. Compounds with values above 0.201 are considered to be potentially carcinogenic[12–18].

Apparent dissociation constants (pK_a) were measured spectrometrically in order to complete characterization of these compounds.

EXPERIMENTAL

N,*N*-Dimethylformamide (DMF), used as a solvent, and tetrabutylammonium perchlorate (TBAP), a supporting electrolyte in all polarographic measurements, and all compounds for preparation of Britton–Robinson (BR) buffer were of analytical grade and were purchased from Fluka (Switzerland). DMF was purified by two-stage vacuum distillation in nitrogen atmosphere[19]. The water content determined by Karl Fischer dead-stop titration was lower than 0.1%. α-Lipoic acid was purchased from Koch–Light Laboratories (Colnbrook, U.K.). Methanol for spectroscopy was obtained from Merck (Darmstadt, Germany).

The studied compounds **1a**–**1i** were prepared according to refs[3–10]. Synthesis of 4*H*-furo[3,2-*b*]pyrrole-2,5-dicarboxylic acid (**1j**) has not been described yet.

4*H*-Furo[3,2-*b*]pyrrole-2,5-dicarboxylic Acid (**1j**)

A solution of sodium hydroxide (5 ml; 5%) was added to a hot solution of methyl 2-cyano-4*H*-furo[3,2-*b*]pyrrole-5-carboxylate[10] (0.95 g, 5 mmol) in ethanol (20 ml) and the reaction mixture was refluxed for 2 h. Then, the reaction mixture was acidified with hydrochloric acid to pH 6.5 and quickly poured onto crushed ice. The precipitate was filtered off and crystallized from ethanol to give **1j** as white powder (0.878 g; 90%), m.p. 350 °C. For $C_8H_5NO_5$ (195.1) calculated: 49.24% C, 2.58% H, 7.18% N; found: 49.38% C, 2.43% H, 7.24% N. ^{1}H NMR (DMSO-d_6): 6.77 s, 1 H (H-6); 7.24 s, 1 H (H-3); 11.80 s, 1 H (NH); 12.72 bs, 2 H (COOH).

DC Polarography

Polarographic measurements were performed on a polarographic analyzer PA 4 in a three-electrode circuit using an XY recorder 4106 (Laboratory Instruments, Prague, Czech Republic). As the indicating electrode, a dropping mercury electrode was used with a dropping time of 3 s and the flow rate 2.27 mg s^{-1} at a mercury column height of 81 cm. As the reference electrode, a saturated calomel electrode (SCE) Radelkis OP 830 (Budapest, Hungary) modified for anhydrous conditions was used. Standard SCE is switched to supporting electrolyte by salt bridge (DMF + 0.15 mol dm^{-3} TBAP) by Beran[20]. As the auxiliary electrode, a standard platinum electrode Radelkis OH 9377 (Budapest, Hungary) was used. All polarographic measurements were carried out at room temperature in nitrogen stream. The concentration of the compounds tested in the polarographic reduction was 0.5 mmol dm^{-3}. The molar ratio analyte : α-lipoic acid (the carcinogenic modulator) ranged from 1.25 : 0.1 to 1.25 : 1.5. The number of electrons involved in the reduction was determined by logarithmic analysis of polarographic curves as log $i_l/(i_d - i_l)$ *vs* *E* plots, where i_d is the diffusion current at the voltage *E* and i_l the limiting current (Tomes analysis) and by Stackelberg[21].

Determination of pK_a

The standard procedure described by Albert and Serjeant[22] was used. Methanolic stock solutions were prepared at a concentration of $2 \cdot 10^{-3}$ mol dm^{-3}. The stock solutions were diluted to 1–5 $\cdot$ 10^{-5} mol dm^{-3} with BR buffer of different pH so that the measured compounds were present as molecular species, partly (pH 2.50–6.00 or 8.30–11.50) or totally (0.1–2 M HCl or NaOH) ionized. Their spectra were recorded on a UV-VIS double-beam spectrometer Camspec M350 (Cambridge, U.K.) in the wavelength range 200–400 nm. The absorption data at one or two wavelengths (before and after isosbestic point) was used for calculation of pK_a values.

RESULTS AND DISCUSSION

All of the tested compounds were dissolved in DMF (with the exception of compound **1j**) and their solutions underwent polarographic reduction in one, two, or three steps. Compounds **1a**–**1c** differ by the presence of methyl groups: compound **1a** is the parent compound, **1b** and **1c** are its mono- and dimethyl derivatives, respectively. These compounds are reduced in a single two-electron step in the range from –2.320 to –2.460 V *vs* SCE, with increasing number of methyl groups, the half-wave potential ($E_{1/2}$) shifts to

TABLE I
Experimental polarographic data of the studied compounds

Compound	$E_{1/2,I}$, V	$E_{1/2,II}$, V	$E_{1/2,III}$, V	$E_{1/2,compl.}$, V[a]	tg α
1a	–2.320	–	–	–1.320	0.0134
1b	–2.400	–	–	–1.275	0.0275
1c	–2.460	–	–	–1.310	0.0110
1d	–2.120	–2.510	–	–1.320	0.0253
1e	–1.850	–2.110	–2.300	–1.275	0.0272
1f	–1.340	–1.910	–	–	–
1g	–2.260	–	–	–1.220	0.0244
1h	–2.390	–	–	–1.250	0.0254
1i	–2.415	–	–	–1.230	0.1040
1j	–	–	–	–	–
2a	–2.410	–	–	–1.260	0.0439
2b	–1.540	–1.980	–	–1.250	0.116

[a] $E_{1/2,compl.}$ is the value of the half-wave potential of complex of studied compounds with α-lipoic acid.

more negative values (Table I). Presence of the phenyl group in **1d** causes a change in the reduction mechanism. Compound **1d** is reduced in two one-electron steps, where $E_{1/2}$ of the first step is by 200 mV more positive than the $E_{1/2}$ of **1a** (Table I). *N*-Methyl derivative **1e** contains chlorine on the phenyl ring. This structural change results in another alteration of the reduction mechanism. From all the studied compounds, only one electron is reduced in three steps, the last step being irreversible. Compound **1f**, a formyl derivative of **1a**, is reduced in two steps whereby $E_{1/2}$ value is by nearly 1 000 mV more positive in comparison with **1a**. Compounds **1g**–**1i** are carboxylic acids differing in the position and number of methyl groups. These compounds are reduced in a single step similarly as compounds **1a**–**1c**, the successive methylation resulting in the shift of $E_{1/2}$ to more negative values. In compounds **1f** and **2b** containing a formyl group, a different reduction mechanism causing shift of the $E_{1/2}$ to more positive val-

TABLE II
UV spectra and pK_a values of the studied compounds

Compound	λ_{max}, nm	log ε	pK_a[a]
1a	297	4.16	9.58 ± 0.07
1b	304	4.04	10.08 ± 0.04
1c	308	3.98	10.46 ± 0.02
1d	334	3.97	10.01 ± 0.03
	350 sh	3.88	
1e	–	–	–
1f	340	3.99	9.80 ± 0.04
1g	282	3.79	4.01 ± 0.04
			9.78 ± 0.07
1h	292	3.98	4.40 ± 0.02
			10.10 ± 0.07
1i	294	3.81	4.81 ± 0.03
			10.36 ± 0.05
1j	–	–	–
2a	292	4.16	10.37 ± 0.06
2b	334	3.95	9.47 ± 0.02

[a] Each of the pK_a value is a mean of three measured values.

ues was observed (Table I). Potential carcinogenic activity of the studied compounds was determined in the presence of α-lipoic acid. The tested compounds exhibited negligible values of tg α, with the exception of compound **2b**. Its tg α value is 0.116 and hence it is assumed to have marginal carcinogenic activity.

Determination of UV absorption maxima, molar absorption coefficients and apparent dissociation constants (pK_a) complete characterization of the tested compounds. The results are summarized in Table II. The absorbances of molecular, partly and fully dissociated, species were measured at 22 °C and their pK_a values were calculated. Methylation of compounds **1b** and **1c** increases their basicity and the presence of the formyl group in **1f** with its negative induction effect reduces its pK_a in comparison with the parent compound **1a**. Presence of phenyl group in compound **1d** is probably responsible for its strong basicity. Methylation of compounds **1h** and **1i** has the same effect as mentioned above, both the compounds appear more basic in comparison with the parent compound **1g**. Compounds **1e** and **1j** are insoluble in methanol.

These results show that methylation of **1a** shifts the $E_{1/2}$ to a more negative value and increases the apparent pK_a, *i.e.* the molecule becomes more basic. On the other hand, compound **1d**, a phenyl derivative of the parent compound **1a**, was reduced at a markedly negative $E_{1/2}$ value. Introduction of a chlorine atom into the benzene ring of **1d** is responsible for the change in the reduction mechanism and the third reducing step observed in **1e** is more positive by 100 mV compared to **1d**. The exchange of the phenyl group in **1d** for the formyl group in **1f** markedly increases acidity of the compound and shifts the $E_{1/2}$ to more positive values. Compound **1f** is the only one of the tested substances that did not interfere and did not form a complex with α-lipoic acid. Compounds **1g**–**1i** are monocarboxylic acids, **1j** is a dicarboxylic acid. Similarly as in the case of compounds **1a**–**1c**, the shift of $E_{1/2}$ of carboxylic acids **1g**–**1i** and **1j** to more negative values is due to their methylation. The introduction of the formyl group into the molecule of **2a** is a probable reason for the reduction of the compund in two one-electron steps, the $E_{1/2}$ of the first polarografic wave of compound **2b** being by 840 mV more positive. Both methylated monocarboxylic acids **1h** and **1i** show higher basicities compared to **1g**. From the point of view of potential carcinogenic activity, and on the basis of determination of the parameter tg α, it can be concluded that the studied compounds do not show carcinogenicity with the exception of **2a**, which possesses a marginal activity.

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