Protonation Sites of Indoles and Benzoylindoles

Francisco J. Hoyuelos,[a] Begoña García*[a] Saturnino Ibeas,[a] María S. Muñoz,[a] Ana M. Navarro,[a] Indalecio Peñacoba,[a] and José M. Leal[a]

Keywords: Acidity function / Protonation / Indole / Benzoylindole / Indomethacine / Acemethacine

Indomethacine (**1**) and acemethacine (**2**) contain the substituents -OCH_3, -CH_2COOH, -CH_2COOCH_2COOH, C-indole and O-benzoyl, which are susceptible to protonation in highly acidic media. To determine the protonation sites and the substituent effects reliably, the dissociation constants of the set of structurally related compounds 1-benzoyl-3-methylindole (**3**), 1-benzoyl-5-methoxyindole (**4**), 1-benzoylindole (**5**), 5-methoxy-2-methylindole (**6**), (2-methylindol-3-yl)acetic acid (**7**) and (5-methoxy-2-methylindol-3-yl)acetic acid (**8**) were investigated in concentrated perchloric acid. The UV/Visible spectral curves were studied by the Hammett, Bunnett–Olsen and excess acidity methods, and the observed medium effects of **1** to **5** were analysed by the vector analysis and excess acidity methods. Acemethacine (**2**) displays two acid–base equilibria with $pK_{SH^+}(i) = -2.3$ and $pK_{SH^+} = -4.2$, corresponding to the ester and amide groups. The benzoylindoles **1**, **3**, **4** and **5** each show a single amide equilibrium $pK_{SH^+} \approx -4.2$, independently of the substituent. Indoles **6**–**8** are stronger bases than benzoylindoles. Substitution of the indole H (NH) by the benzoyl group strongly reduces the N basicity, and increases the O basicity with protonation of the CO group. The solvation parameters, $m^* = 0.70$ for benzoylindoles and $m^* = 1.3$ for indoles, reveal greater charge location and less solvation requirements in the latter.

Introduction

Indomethacine and acemethacine are nonsteroidal anti-inflammatory drugs widely used in medicinal chemistry. Indomethacine, [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid (**1**), first synthesized in the early 1960s,[1] is used as an analgesic in the treatment of rheumatoid arthritis and other degenerative diseases, gout and acute musculoskeletal disorders; it has also been used as an antipyretic to lower febrile symptoms. Appearance of side effects has limited its use and prompted the pharmaceutical industry to synthesize other parent molecules with similar action and better tolerance by patients; acemethacine (**2**), obtained from indomethacine by hydroxyacetic acid esterification, may enable the achievement of such objectives. Indomethacine, a metabolite of acemethacine,[2] continues to be a reference for therapeutic activity.In addition to basic hydrolysis of indomethacine[3] and acemethacine,[3d] acidic hydrolysis is also feasible; the close similarity of their UV/Visible spectral curves makes it difficult to distinguish them from one another and to determine their properties, so study was extended to the set of structurally related compounds 1-benzoyl-3-methylindole (**3**), 1-benzoyl-5-methoxyindole (**4**), 1-benzoylindole (**5**), 5-methoxy-2-methylindole (**6**), (2-methylindol-3-yl)acetic acid (7), and (5-methoxy-2-methylindol-3-yl)acetic acid (**8**) (Scheme 1). Knowledge of acid–base behaviour is often crucial to interpret pharmacokinetics and pharmacodynamics, and to determine hydrolysis mechanisms,[4] so in this work the protonation reaction of the set of compounds and the substituent effects has been undertaken, showing the existing relationships among them.

The compounds investigated contain a number of weakly basic substituents susceptible to partial or full protonation, such as -OCH_3, -CH_2COOH, -CH_2COOCH_2COOH, indole and benzoyl. Except for acemethacine (**2**), which displays two acid–base equilibria ($pK_{SH^+}(i)$ and pK_{SH^+}) the other benzoylindoles each manifest a single substituent-independent pK_{SH^+} value. The protonation equilibria of these substrates (S, indole or benzoylindole) can be represented in the form [Equation (1)]

$$SH^+ \overset{K_{SH^+}}{\rightleftharpoons} S + H^+ \quad (1)$$

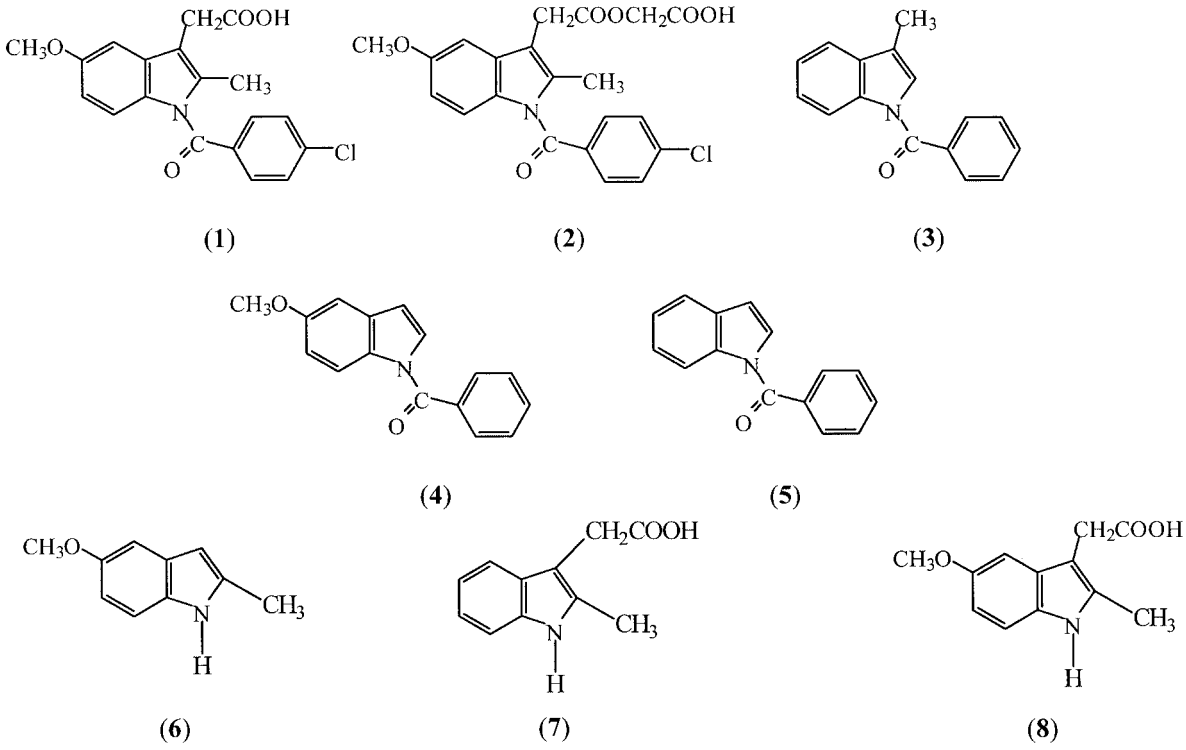
and the equilibrium constants in the form [Equation (2)]:

$$pK_{SH^+} = \log I - \log c_{H^+} - \log\left(\frac{\gamma_S \gamma_{H^+}}{\gamma_{SH^+}}\right) \quad (2)$$

where c_{H^+} is the hydrogen ion concentration, $I = c_{SH^+}/c_s$ represents the ionization ratio, and the last term contains the activity coefficients ratio. Ionization ratios can be exper-

[a] Universidad de Burgos, Departamento de Química, 09001 Burgos, Spain
E-mail: begar@ubu.es

 DOI: 10.1002/ejoc.200400434



Scheme 1. Indomethacine (**1**), acemethacine (**2**), 1-benzoyl-3-methylindole (**3**), 1-benzoyl-5-methoxyindole (**4**), 1-benzoylindole (**5**), 5-methoxy-2-methylindole (**6**), (2-methylindol-3-yl)acetic acid (**7**), and (5-methoxy-2-methylindol-3-yl)acetic acid (**8**).

imentally determined by measuring the observed variation with medium acidity of a particular property of SH^+ in relation to that of S; the most commonly used properties include UV/Visible and ^1H and ^{13}C NMR characteristics.[5] If the absorbance values for the unprotonated (A_S) and the protonated (A_{SH^+}) forms are measurable, then the ionization ratios can be evaluated by Equation (3):

$$I = (A_{SH}{}^+ - A)/(A - A_S) \quad (3)$$

where A stands for the absorbance at intermediate acidities. The pK_{SH^+} values in highly acidic media can then be evaluated by using the following functions and equations:

a) The Hammett equation: although Equation (2) is thermodynamically exact, evaluation of the unknown activity coefficients ratio term constitutes a major difficulty. The first researcher to tackle this problem was Hammett,[6] who defined the H_0 acidity function as Equation (4).

$$H_0 = -\log a_{H^+} \cdot \gamma_B/\gamma_{BH^+} \quad (4)$$

B is the set of primary nitroanilines that serve as reference bases and are susceptible to protonation in strongly acidic media. Although Hammett envisaged H_0 as being applicable to all weak bases S, it was soon realized that H_0 was not valid for all types of weak bases.[7] A detailed analysis of the many available acidity functions[5,6,8–12] suggests that the cancellation assumption of the activity coefficients ratio term ($\gamma_B/\gamma_{BH^+} = \gamma_S/\gamma_{SH^+}$) is feasible only for a reduced set of bases with the same functional group[7,9,13] and in the same acidic solvent;[14] in such a case, $m = 1$ in Equation (5),[6,15] so the closer m tends to unity, the more accurate the pK_{SH^+} values that can be deduced. It has been demonstrated that the acidity functions H_Y are linearly related with H_0;[16] hence, Equations (2) and (4) result in Equation (5):

$$\log I = -mH_Y + pK_{SH^+} \quad (5)$$

The H_Y function depends on: i) the particular base (Y), ii) the solvent, iii) the mineral acid, and iv) the temperature range, and measures the solution ability to protonate the base Y relative to an ideal 1 M solution; in dilute acid $-mH_Y$ reduces to $-mp$H. By a process similar to that of Chandler and Lee,[17] rearrangement of Equations (3) and (5) gives Equation (6).

$$A = \frac{A_S - A_{SH^+}}{1 - 10^{-mH_Y + pK_{SH^+}}} + A_{SH^+} \quad (6)$$

The m, A_S, A_{SH^+} and pK_{SH^+} parameters can be evaluated properly by an iterative fitting of Equation (6) to the experimentally measured A–H_Y data pairs; for this work a set of acidity functions for perchloric acid in water and 25 °C was required. To overcome the difficulty of the cancellation assumption, Bunnett–Olsen,[18] Marziano[19] and Cox–

Yates[20] have suggested other different proposals, which are in fact equivalent as shown below.

i) The Bunnett–Olsen Method (BOM)[18]

This is a general method based on the linear free energy relationship (LFER)[Equation (7)], where the sum (H_0 + $\log C_{H^+}$) plays the role of an acidity function that drops to zero in diluted acidic media.

$$\log I + H_0 = \varphi\,(H_0 + \log C_{H^+}) + pK_{SH^+} \tag{7}$$

Equation (7) provides the pK_{SH^+}values as the intercept, and φ as the slope parameter. According to this LFER, only one of the available set of acidity functions described the protonation of weak bases well; this method has been extensively used by Levi et al.,[21] Edward–Wong[22] and Barnett–O'Connor.[23] Rearrangement of Equations (3) and (7) results in Equation (8).

$$A = \frac{A_S - A_{SH^+}}{1+10^{H_0(\phi-1)+H_0\log C_{H^+}+pK_{SH^+}}} + A_{SH^+} \tag{8}$$

Fitting of Equation (8) to the experimental data pairs by the above iteration procedure enables the φ, A_S, A_{SH^+} and pK_{SH^+} parameters to be evaluated.

ii) The Excess Acidity Method (EAM)

The cancellation assumption is eliminated altogether in the excess acidity method (while this is also named the Marziano–Cimino–Passerini and the Cox–Yates method, this is unfortunate since both are the same and the term "excess acidity method" is preferred). A detailed survey of this method has appeared recently.[24] In the first order (or linear) approach the activity coefficients of the weak bases are assumed to be directly related to each other in the form, see Equation (9).

$$\log\left(\frac{\gamma_S\cdot\gamma_{H^+}}{\gamma_{SH^+}}\right) = m^*\log\left(\frac{\gamma_B\cdot\gamma_{H^+}}{\gamma_{BH^+}}\right) = \log\frac{K_{BH^+}^{H_2O}}{K_{BH^+}} = m^*\,X \tag{9}$$

B is a standard or hypothetical weak base[20] and $K_{BH^+}^{H_2O}$ and K_{BH^+} are the dissociation constants of a protonated base in strong acid medium and in water, respectively, from which the excess acidity function (term first used by Perrin)[25] X can be defined. With Equation (9) in mind, Equation (2) is converted to Equation (10).

$$\log I - \log c_{H^+} = m^*\,X + pK_{SH^+} \tag{10}$$

Equations (9) and (10) were originally formulated by Marziano et al.,[19a] who abbreviated the activity coefficient ratio term for B as M_C [Equation (9)] and the slope as n, later n_{ij}[19d] [Equation (10)]. Inclusion of the parameter m^* removes the need to distinguish the behaviour of the acidity function for different substrates.[20] In strongly acidic solutions, the substrate basicity relies both on pK_{SH^+} and on m^*. The need for two parameters to describe the protonation equilibria stems from the stabilization of SH^+ by internal delocalization of the cationic charge and by solvation effects. The parameter m^* measures the ability of a set of protonated bases to become stabilized by solvation, especially by H-bonding, and accounts for the sensitivity of the equilibrium to the large changes in the acidity of the medium needed to complete the protonation.[26–28] Thus, m^* = 0 represents the upper limit of the solvation requirements on this scale (in water, H_3O^+ with m^* = 0 has the highest solvation requirements), whereas higher m^* values denote weaker solvation; m^* = 1 corresponds to the solvation of primary aniline bases,[20] while the highest m^* values (ca. 2.0) have been observed for protonation of hydrocarbon bases, where internal delocalization of the positive charge is most important and solvation by H-bonding becomes negligible.[29] The function X, defined at a given temperature for a set of bases in a particular acid (sulfuric,[20] perchloric,[20] hydrochloric[30] and hydrobromic[30]), represents the difference between the observed acidity and that of the system if it were to behave ideally.[31] By a procedure similar to that used for Equations (6) and (8), Equation (11) results from Equations (3) and (10).

$$A = \frac{A_S - A_{SH^+}}{1+\left(\frac{c_{H^+}}{K_{SH^+}}\right)10^{m^*X}} + A_{SH^+} \tag{11}$$

Fitting of Equation (11) to the experimentally determined data pairs by the iteration described enables the m^*, A_S, A_{SH^+} and K_{SH^+} parameters to be evaluated. Use of Equations (6), (8) and (11) requires knowledge of the absorbance A as a function of c_{H^+}.

The 1–φ (B–O) and m^* values are all roughly equivalent, although they are defined in quite different ways. For sulfuric acid, a plot of –(H_0 + $\log c_{H^+}$) against X is accurately linear (correlation coefficient 0.9996) over the 0–99.5 wt% concentration range, slope 0.983 (for perchloric acid, slope 0.890 and correlation coefficient 0.9920, over the 1–70 wt% concentration range). Noto et al.[32] showed that the equivalence between the B–O and the EA methods is fulfilled for acid solutions up to 45 % perchloric acid. Outside this range significant differences must be observed in the dissociation constants estimates of the three different method. –(H_0 + $\log c_{H^+}$) is identified with X_0,[20] but in fact X_0 is an unrealistic function since it is obtained only from primary aromatic amines. Often, the free energy formulations have a number of important advantages over acidity function treatments.[29] In this work, thepK_{SH^+} values deduced by the

Excess Acidity method differed slightly from those obtained by B–O, a feature ascribable to the weakness of the bases studied, which required perchloric acid concentrations far above 45 % (6.02 M). Actually, the excess acidity function X and the Cox–Yates Equation (11) are most widely used.

Correction of Medium Effects

Equations (6), (8) and (11) do not work properly when the spectral curves exhibit medium effects, characterized by a shift of the absorbance bands upon increasing acidity.[6b,13a,33] Several methods for correction of these effects have been put forward. The methods of Flexser et al.,[34] and Dunn et al.[35] have historical interest, and those of Katritzky et al.[13e,36] and Yates–McClelland[16] leave space for arbitrariness. The method of Davis–Geissman[37] and the modification by Stewart–Yates[38] need identification of the S and SH^+ characteristic wavelengths, often difficult due to medium effects. The Cox–Yates and vector analysis methods have been shown to be most efficient and are widely used.[26–28,39]

a) The Cox–Yates Method

This method, developed by Cox[31,40] from an earlier approach,[41] involves introduction of the excess acidity function (or medium parameter) X [Equation (9)] and enables the analysis of a large number of spectral curves over a wide wavelength range, yielding consistent and reliable results. Thermodynamic pK_{SH^+} values can only be determined from extrapolation to infinite dilution of the data pairs measured in aqueous solutions, see Equation (10). Experimental and theoretical aspects of this issue have been treated extensively.[20,27,28,39–43]

b) The Vector Analysis Method

Medium effects have been treated successfully by characteristic vector analysis (CVA) in the protonation of amides,[28c,42d] hydroxamic acids,[27,28a] pyrimidines,[28d] hydrazones[44] and ketones.[45] This method reduces the experimentally determined absorbance readings into the minimum number of independent components capable of reproducing the spectral curves, from which reliable ionization ratios can be deduced.[46] The absorbance readings A, measured at n different acidity levels and r wavelengths, were arranged into an $(n \times r)$ matrix array; if p independent components are needed to reproduce the experimental readings, then the absorbance values at every wavelength λ can be expressed for each c_{H^+} value by the set of equations. [Equation (12)].

$$A_r = \bar{A}_r + c_1 \cdot \mathbf{v}_{1r} + c_2 \cdot \mathbf{v}_{2r} + \ldots + c_p \cdot \mathbf{v}_{pr} \quad (12)$$

where $\bar{A}_r$ is the average absorbance corresponding to a particular wavelength for the n different c_{H^+} values, $\mathbf{v}_{pr}$ are the characteristic vectors, and c_p the weighing factors (or fitting coefficients). It was demonstrated that in practice only two characteristic vectors are sufficient to describe the total effect, the first vector ($\mathbf{v}_{1r}$) representing the protonation effect caused by the change in the medium acidity, and the second ($\mathbf{v}_{2r}$) the medium effects upon protonation.[22,25] Values for I and pK_{BH^+} can be determined from the variation of c_1 with medium acidity; if $c_{1,B}$ and c_{1,BH^+} are the coefficients for the fully unprotonated and the fully protonated base, then the ionization ratios can readily be determined as in Equation (13).

$$I = \frac{(c_{1,S} - c_1)}{(c_1 - c_{1,SH^+})} \quad (13)$$

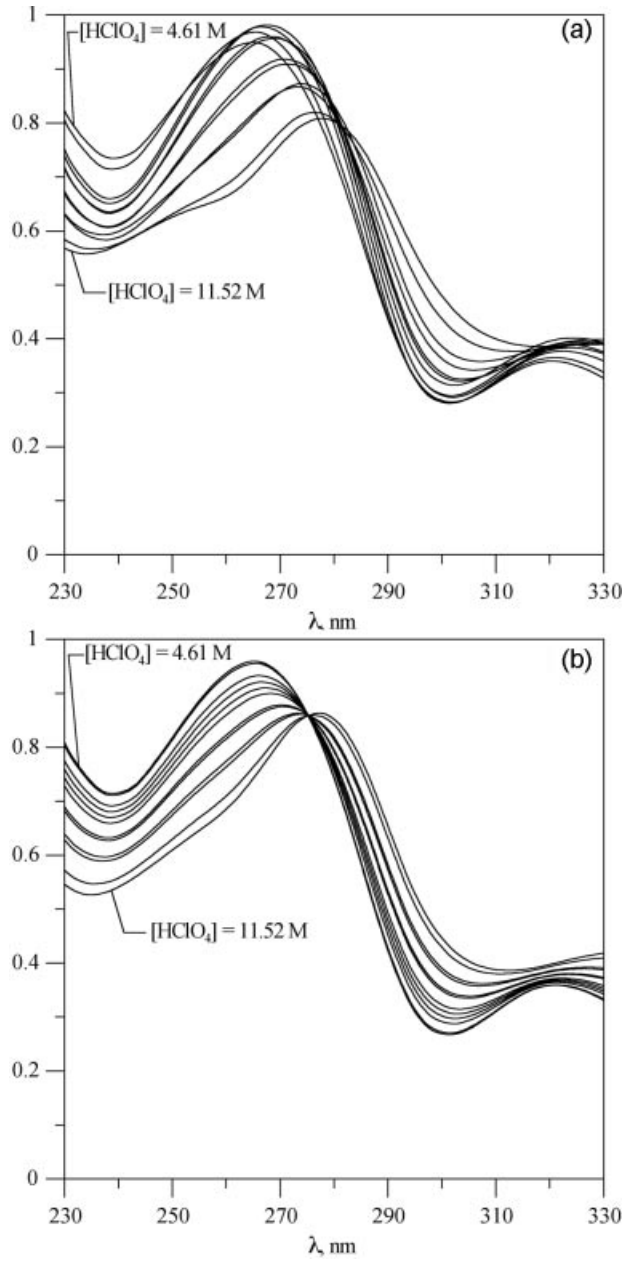


Figure 1. Sets of UV spectral curves of indomethacine in perchloric acid media recorded: a) before, and b) after application of vector analysis.

c_1 being the coefficients at intermediate extents of protonation. Once the c_1 characteristic vectorsare determined as a function of c_{H^+}, accurate pK_{SH^+} values can be deduced through substitution of A, A_S and A_{SH^+} by c_1, $c_{1,S}$and c_{1,SH^+} in Equations (6), (8) and (11). This methodby construction leads to perfect isosbestic points, but the resulting data are not more accurate per se.

Results and Discussion

The medium effects observed (Figure 1a, Figure 2a, Figure 3a, Figure 4a and Figure 5a) in the absorbance spectra ofbenzoylindoles **1** to **5** were corrected by the procedures described (Figure 1b, Figure 2b, Figure 3b, Figure 4b and Figure 5b). Acemethacine displayed two clearly differentiated acid–base equilibria, p$K_{SH^+}(i)$ (between 0.92 and 8.76 M $HClO_4$ with an isosbestic point at 257 nm) andpK_{SH^+}(between 9.22 and 11.52 M $HClO_4$ with an isosbestic point at 272 nm)(Figure 2b), whereas the other compounds each displayed only a single constant pK_{SH^+}. Indomethacine shows an isosbestic point between 4.6 and 11.5 M; the spectra below 4.6 M remained unchanged. Table 1 lists the c_1(i) and c_1 coefficients at different c_{H^+} values for all five substrates over the whole titration acidity range. The spectral curves of indoles **6**, **7** and **8** were quite

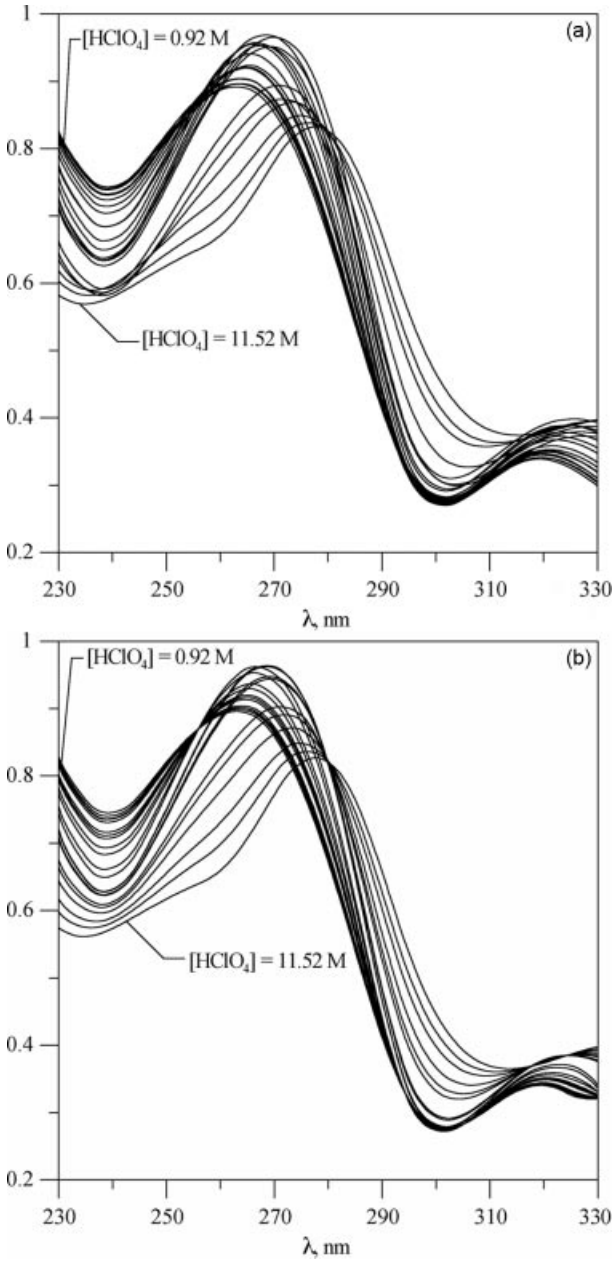


Figure 2. Sets of UV spectral curves of acemetacine in perchloric acid media recorded: a) before, and b) after application of vector analysis.

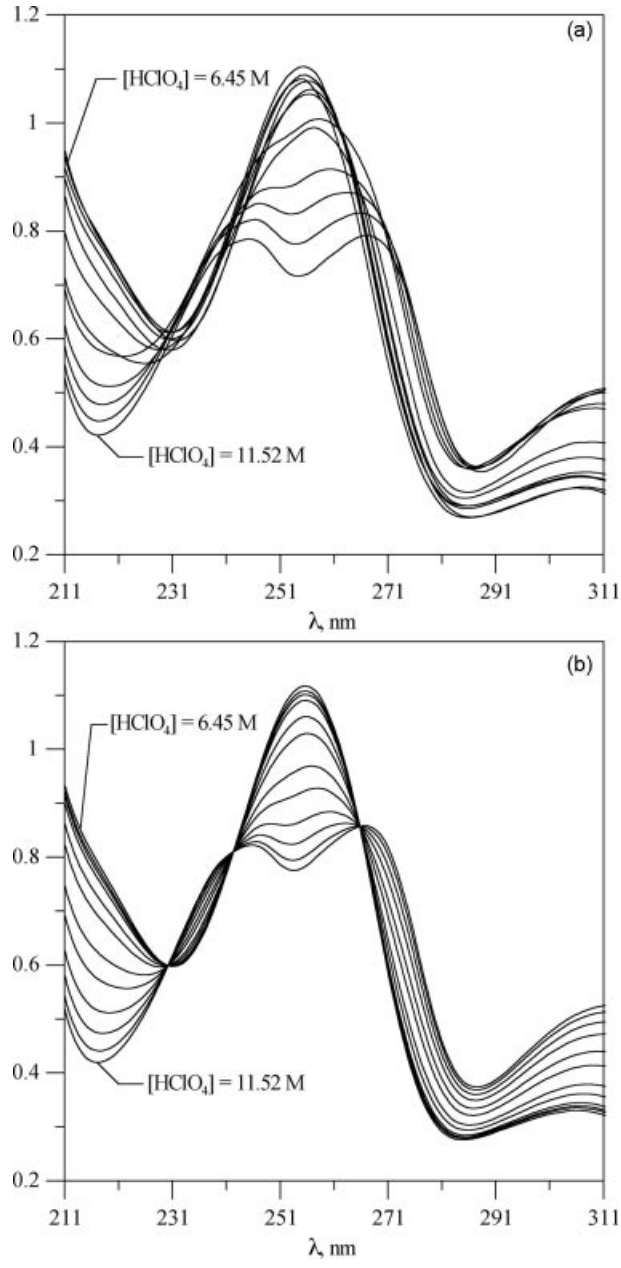


Figure 3. Sets of UV spectral curves of 1-benzoyl-3-methylindole in perchloric acid media recorded: a) before, and b) after application of vector analysis.

similar (Figure 6), the sharp and well defined isosbestic points simplifying the calculations.

Application of Equation (6) requires use of the acidity functions H_Y, the choice of which depends on the particular substrates. For benzoylindoles **1** to **5**, the H_I function in 0.1 to 6.0 M perchloric acid defined by Hinman–Lang[47] for alkylindoles was insufficient to describe full protonation; substitution of H_Y by H_A[10,11] or H_0[8d,48] into Equation (6) gave m values far from unity, so the resulting constants were not reliable. Table 2 lists the results deduced with Equations 8 and 11.

To gain a deeper insight into the protonation and further decomposition of indomethacine and acemethacine, the

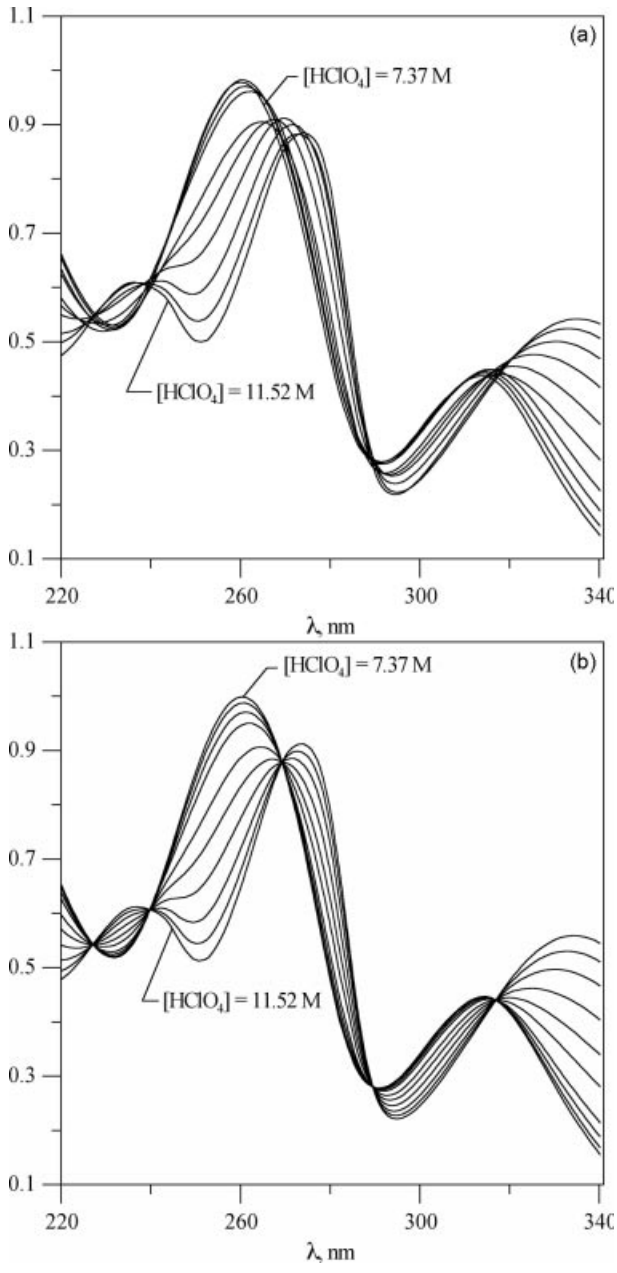


Figure 4. Sets of UV spectral curves of 1-benzoyl-5-methoxyindole in perchloric acid media recorded: a) before, and b) after application of vector analysis.

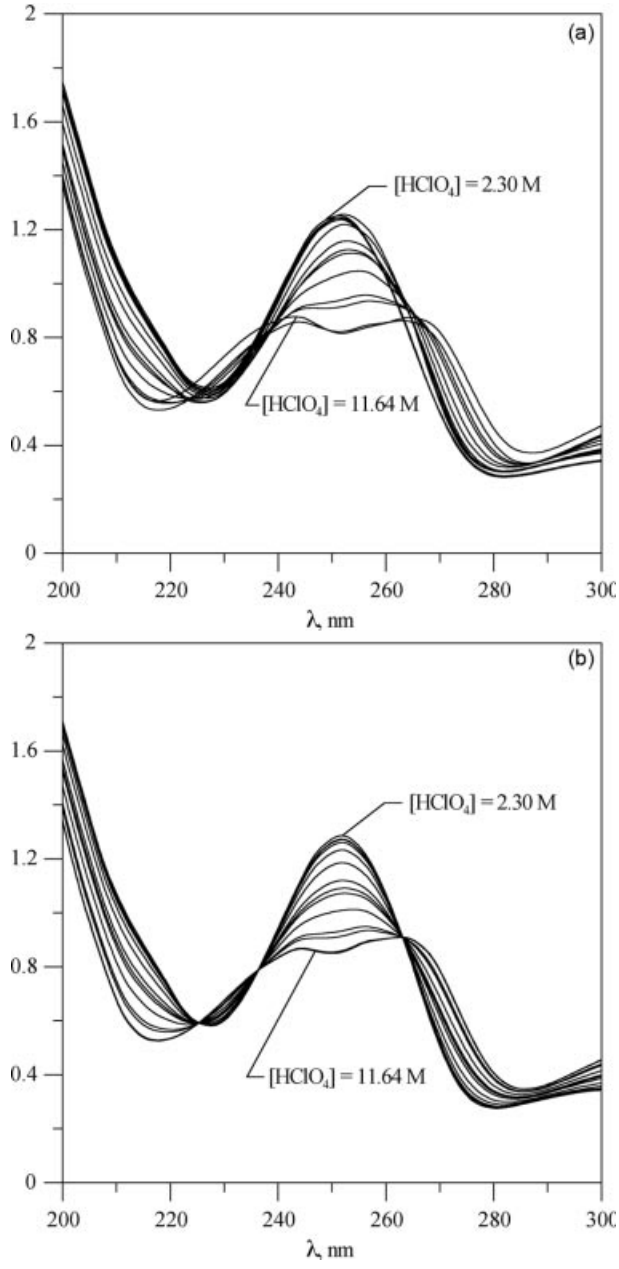


Figure 5. Sets of UV spectral curves of 1-benzoylindole in perchloric acid media recorded: a) before, and b) after application of vector analysis.

pK_{SH^+} values of the structurally related indoles **6** to **8** were also evaluated with the H_I and H_0 functions; only H_I fulfilled the cancellation assumption [$m \approx 1$, in Equation (6)] (Table 3). Indoles are very weak bases, so protonation occurs outside the pH scale.[49] In concentrated perchloric acid indoles protonate at position 3 (or the β position), forming conjugated acids with the charge distributed mainly between the N and C 2 (or α C) sites[47,50] (Scheme 2).

Indole **6** undergoes protonation between 0.003–3.0 M $HClO_4$, so pH could be used as an acidity measure in the diluted acid limit; the good agreement between pH and H_I, enabled the full protonation curve to be drawn (Figure 7) and Equation (6) to be applied by substitution of H_Y by H_I

Table 1. c_1 coefficients determined by vector analysis at different acid molarities (M): indomethacine (**1**), acemetacine (**2**), 1-benzoyl-3-methylindole (**3**), 1-benzoyl-5-methoxyindole (**4**), and 1-benzoylindole (**5**).

1		**2**		**2**		**3**		**4**		**5**	
M	c_1	M	c_1 (i)	M	c_1	M	c_1	M	c_1	M	c_1
4.61	0.247	0.92	–0.345	9.22	0.357	6.45	–0.341	7.37	0.388	4.61	0.326
5.53	0.245	2.30	–0.297	9.45	0.364	6.91	–0.319	7.83	0.359	5.07	0.303
6.91	0.231	2.77	–0.259	9.68	0.291	7.37	–0.303	8.30	0.313	5.53	0.300
8.76	0.148	3.23	–0.251	9.91	0.275	7.83	–0.277	8.76	0.255	5.99	0.281
9.22	0.102	3.69	–0.208	10.14	0.073	8.30	–0.206	9.22	0.105	8.30	0.233
9.45	0.057	5.70	–0.069	10.37	0.007	8.76	–0.131	9.68	–0.028	8.76	0.153
9.68	0.011	5.53	–0.022	10.60	–0.117	9.22	0.015	10.14	–0.174	9.22	0.039
10.14	–0.102	5.99	0.023	11.06	–0.29	9.68	0.120	10.60	–0.315	9.45	–0.011
10.37	–0.124	6.91	0.152	11.29	–0.41	10.14	0.245	11.06	–0.416	9.68	–0.044
10.60	–0.250	7.37	0.247	11.52	–0.555	10.60	0.334	11.52	–0.493	10.14	–0.153
10.83	–0.282	8.30	0.455			11.06	0.405			10.60	–0.285
11.29	–0.446	8.76	0.568			11.52	0.451			10.83	–0.318
11.52	–0.520									11.29	–0.419
										11.52	–0.413

Table 2. Protonation parameters determined by vector analysis with use of different equations. Mean pK_{SH^+} values determined from individual values of table: indomethacine (**1**), acemetacine (**2**), 1-benzoyl-3-methylindole (**3**), 1-benzoyl-5-methoxyindole (**4**), and 1-benzoylindole (**5**).

Function (Equation)	$c_{1,S}$	c_{1,SH^+}	Parameter	pK_{SH^+}	χ^2	average pK_{SH^+}
1						
H_0[a] [see Equation (8)]	0.25 ± 0.01	–0.9 ± 0.1	φ = –0.53 ± 0.05	–3.8 ± 0.2	0.00023	
X[b] [see Equation (11)]	0.25 ± 0.01	–1.0 ± 0.2	m^* = 0.60 ± 0.06	–4.2 ± 0.2	0.0023	–4.0 ± 0.2
2 (i)						
H_0[a] [see Equation (8)]	–0.39 ± 0.01	3 ± 2	φ = 0.85 ± 0.05	–2.0 ± 0.2	0.00013	
X[b] [see Equation (11)]	–0.33 ± 0.01	0.93 ± 0.07	m^* = 0.35 ± 0.04	–2.0 ± 0.06	0.00058	–2.0 ± 0.1
2						
H_0[a] [see Equation (8)]	0.6 ± 0.2	–0.9 ± 0.5	φ = 0.4 ± 0.3	–4 ± 1	0.00191	
X[b] [see Equation (11)]	0.62 ± 0.05	–1.2 ± 0.3	m^* = 0.60 ± 0.03	–4.3 ± 0.2	0.00189	–4.2 ± 0.5
3						
H_0[a] [see Equation (8)]	–0.37 ± 0.01	0.49 ± 0.02	φ = 0.38 ± 0.03	–3.8 ± 0.1	0.0001	
X[b] [see Equation (11)]	–0.36 ± 0.01	0.50 ± 0.02	m^* = 0.71 ± 0.04	–4.2 ± 0.2	0.0001	–4.0 ± 0.2
4						
H_0[a] [see Equation (8)]	0.44 ± 0.02	–0.56 ± 0.02	φ = 0.39 ± 0.04	–4.0 ± 0.2	0.00014	
X[b] [see Equation (11)]	0.44 ± 0.02	–0.58 ± 0.03	m^* = 0.71 ± 0.05	–4.4 ± 0.2	0.00014	–4.2 ± 0.2
5						
H_0[a] [see Equation (8)]	0.31 ± 0.01	–0.49 ± 0.03	φ = 0.41 ± 0.05	–3.9 ± 0.2	0.00033	
X[b] [see Equation (11)]	0.31 ± 0.01	–0.51 ± 0.04	m^* = 0.68 ± 0.06	–4.3 ± 0.3	0.00033	–4.1 ± 0.3

[a] Refs.[8d,48] [b] Ref.[20]

or pH depending on the high or low acidity region; the pH scale and the excess acidity scale, X, do not correspond well in dilute solution,[51] so X is inadequate to describe the full protonation range. Moreover, combination of pH and H_0 in Equation (8) does not give self-consistent parameters. Indoles **7** and **8** undergo protonation in 0.5–7.5 M perchloric acid and Equations (6), (8) and (11) apply satisfactorily.

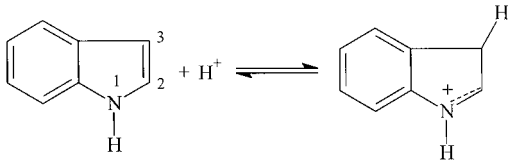
Table 2 and Table3 list the $c_{1,S}$, c_{1,SH^+} and pK_{SH^+} values for benzoylindoles **1**–**5**, A_S, A_{SH^+} and pK_{SH^+} for indoles **6**–**8**, and the m, φand m^* parameters evaluated by iteration of Equations (6), (8) and (11), with the c_1-H_Y and c_1-X or A-H_Y and A-X data pairs, A being the indole absorbance.

Comparison between benzoylindoles **1** to **5** and indoles **6** to **8** affords the following conclusions: a) benzoylindoles undergo acid-catalysed decomposition (not shown), whereas indoles remain stable in acidic medium, b) in contrast to indoles (Table 4) substituents do not affect the acid–base behaviour of benzoylindoles (Table 2), c) benzoylindoles are weaker bases than indoles; for instance, pK_{SH^+} = –4.0 for indomethacine (**1**), whereas pK_{SH^+} = –2.1 for the corresponding (5-methoxy-2-methylindol-3-yl)acetic acid (**8**), d) the set of spectral curves of **6** to **8** exhibited well defined isosbestic points (Figure 6), whereas **1** to **5**, with the indole N in the amide group, displayed noticeable medium effects (Figure 1a, Figure 2a, Figure 3a, Figure 4a and Figure 5a). No experimental evidence for protonation of the methoxy group in concentrated perchloric acid was found. The methoxy group ring gives a weaker base than indole

Table 3. Protonation parameters determined by different equations, with mean pK_{SH^+} values determined from individual values of the table: 5-methoxy-2-methylindole (**6**), (2-methylindol-3-yl)acetic acid (**7**) and (5-methoxy-2-methyl)indol-3-ylacetic acid (**8**).

Function (Equation)	A_s	A_{1,SH^+}	Parameter	pK_{SH^+}	χ^2	average pK_{SH^+}
6						
H_I[c] [see Equation (6)]	1.302 ± 0.005	0.175 ± 0.007	m = 1.33 ± 0.06	0.17 ± 0.02	0.00026	0.17±0.02
7						
H_I[c] [see Equation (6)]	1.46 ± 0.01	0.15 ± 0.02	m = 0.88 ± 0.04	–2.5 ± 0.1	0.00025	
H_0^a [see Equation (8)]	1.477 ± 0.008	0.166 ± 0.006	φ = –0.48 ± 0.04	–2.25 ± 0.05	0.00017	–2.30±0.1
X[b] [see Equation (11)]	1.467 ± 0.007	0.159 ± 0.006	m* = 1.22 ± 0.04	–2.14 ± 0.05	0.00015	
8						
H_I[c] [see Equation (6)]	1.17 ± 0.01	0.19 ± 0.01	m = 0.99 ± 0.05	–2.3 ± 0.1	0.00023	
H_0^a [see Equation (8)]	1.19 ± 0.02	0.182 ± 0.009	φ = –0.9 ± 0.1	–2.1 ± 0.1	0.00034	–2.08±0.1
X[b] [see Equation (11)]	1.18 ± 0.01	0.178 ± 0.009	m* = 1.39 ± 0.08	–1.83 ± 0.09	0.00029	

[a] Refs.[8d,48] [b] Ref.[20] [c] Ref.[4]



Scheme 2.

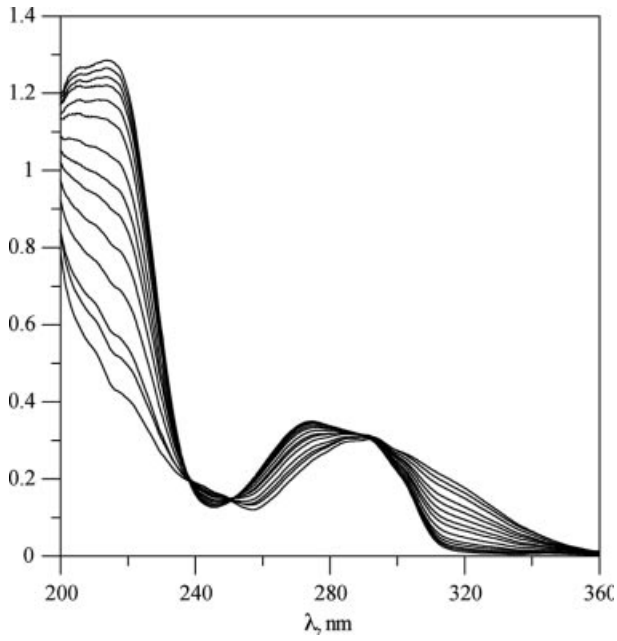


Figure 6. Sets of UV spectral curves of 5-methoxy-2-methylindole (**6**).

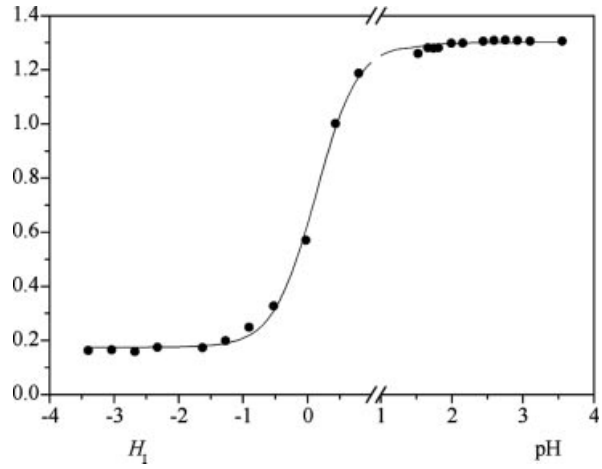
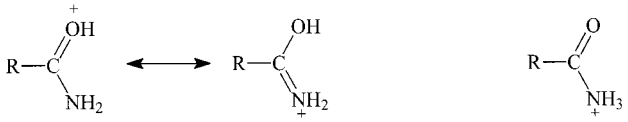


Figure 7. Absorbance data (λ = 215.22 nm) of 5-methoxy-2-methylindole (**6**) in aqueous perchloric acid solution at 25 °C according to Equation (6).

when bound to an aromatic (for instance, pK_{SH^+} methoxy benzene = –6.54)[52] Table 3 shows that indoles **7** and **8** have similar acidity strengths; the difference in reactivity relative to **6** is due to the acetyl electron-withdrawing effect, which lowers the indole ring basicity. Although the pK_{SH^+} values of indole and five derivatives reported by Berti et al.[53] disagreed significantly with those reported by Hinman–Lang,[47] they in fact yielded the same sequence basicity. Table 4 lists the substituent effects on indoles' basicities. The significant differences are functions of the substituent and ring site; donor substituents such as 2-methyl and 5-methoxy strongly increase the basicity, as demonstrated by comparison of the pK_{SH^+} values of indole and 2-methylindole with that of 5-methoxy-2-methylindole (**6**), and of that of indol-3-ylacetic acid with that of 5-methoxy-2-methyl indole-3-acetic (**8**). The finding that 2-methylindole is a stronger base than indole can be explained in terms of cation stabilization by electron release from the methyl group. The spectral curves of benzoylindoles displayed medium effects similar to those observed in the protonation of the carbonyl group.[28c,54–59] The NMR spectra of amides in strongly acid solutions are consistent with *O*-protonation;[60] the main factor contributing to the stability of the *O*-protonated form, the O–C–N π delocalization, was absent for the *N*-protonated form (Scheme 3), although the latter has been observed as a secondary species at low concentration.[61] The close similarity of the spectrophotometric behaviour of benzoylindoles and amides and their observed hydrolyses reveal that although indoles are stronger bases than benzoylindoles, protonation in benzoylindoles preferentially occurs at the O(CO) site rather than at the indole group.

Table 4. Acidity constants (pK_{SH^+}) for different indoles.

Indole	pK_{SH^+}
5-methoxy-2-methylindole (**6**)	+0.17
(2-methylindol-3-yl)acetic acid (**7**)	–2.30
(5-methoxy-2-methylindol-3-yl)acetic acid (**8**)	–2.08
Indole	–2.43[47]
	–2.5[50]
	–2.5[53]
	–2.8[54c]
1-methylindole	–2.32[47]
2-methylindole	–0.28[47]
3-methylindole	–4.55[53]
	–4.0[54c]
2,3-dimethylindole	–1.5[47,51]
3-formylindole	–1.36[52c]
(indol-3-yl)acetic acid	<–5[55]
3-acetylindole	–1.5[54c]



Scheme 3.

It can be concluded, then, that the presence of the CO group strongly decreases the indole basicity and increases the O-basicity.[62] Acemethacine, with two acid–base equilibria, represents a particular situation. Since the methoxy group undergoes no protonation reaction, the pK_{SH^+} value of –4.2 for acemethacine (similar to other benzoylindoles), should correspond to protonation of the O(CO) amide, whereas the constant $pK_{SH^+}(i)$ = –2.3 should be ascribed to O(CO) protonation of the 3-acetoxy group with further hydrolysis to produce indomethacine, observed by ^1H NMR, ^{13}C NMR and mass GC.[63] The appearance of medium effects (Figure 2a) and the two well differentiated equilibria (Figure 2b) is also consistent with protonation of the two CO oxygens.

Protonation equilibria are fully defined in terms of pK_{SH^+} and either φ (Bunnett–Olsen method) or m^* (excess acidity method); φ represents the response of the equilibrium to the change in the medium (i.e., increase in c_{H^+} and subsequent decrease of a_{H_2O}) and is a measure of the effects caused by the changes of hydration.[18,21,42b] If primary anilines are taken as a reference (φ = 0),[18] then positive φ values should be expected for bases with higher solvation requirements, and negative ones when hydration of SH^+ is comparatively low. The parameter m^*, characteristic of the behaviour of the set of bases, reflects the stabilization of the protonated base both by internal delocalization of the cationic charge and by solvation, especially by H-bonding.[29] Cox–Stewart[64] suggested a change of ±0.1 m^* units as a measure of homogeneous behaviour of the bases under consideration; thus, m^* = 0 for H_3O^+ denotes the highest solvation requirements. Other values are: m^* = 0.18 for ethers,[29,65] 0.4–0.6 for *O*-protonation of amides,[20] m^* = 1 for primary amines,[20,66,67] 0.56 for hydroxamic acids,[27] and 1.4 for tertiary aromatic amines.[29] The highest m^* values (ca. 2.0) are observed for protonation of hydrocarbon bases, where internal delocalization of the positive charge is most important and solvation through H-bonding becomes negligible.[20,29,30,65]

The difference between benzoylindoles, m^* = 0.70 (Table 2), and indoles, m^* = 1.3 (Table 3), stems from the reduction of H-bonding upon substitution of H by the benzoyl group, which implies strong indole charge localization and weaker solvation requirements. The close m^* values for indoles and amines, and the difference with the m^* values for *C*-protonation (ca. 2.0), support the charge being located on nitrogen, as shown in Scheme 2. Solvation requirements and H-bonding are less for benzoylindoles than for amides and benzohydroxamic acids, and higher than amine protonation, as the corresponding m^* values denote.

Experimental Section

Reagents: Indomethacine (**1**), acemethacine (**2**), 1-benzoyl-3-methylindole (**3**), 1-benzoylindole (**5**), 5-methoxy-2-methylindole (**6**), (2-methylindol-3-yl)acetic acid (**7**) and (5-methoxy-2-methylindol-3-yl)acetic acid (**8**) were commercially available (Sigma–Aldrich) and were used without further purification. 1-Benzoyl-5-methoxyindole (**4**) was synthesized as described elsewhere,[69] the resulting HPLC purity being 99 %; ^1H, ^{13}C NMR and mass spectra were consistent with the quoted purity. Since the substrates were only sparingly soluble in pure water, the experiments to evaluate the solvent effect on the equilibrium constants were carried out in 1 % v/v acetonitrile/H_2O aqueous-organic solvents.

Methods: The acid solutions were prepared by careful addition of the appropriate amounts of commercial $HClO_4$ to a 100 mL flask containing doubly distilled deionized water, and the equilibria were investigated at 25 °C by the UV spectrophotometric technique. Because of hydrolysis of the benzoylindoles (**1** to **5**), with a constant of around 0.001 s^{-1} at high perchloric acid concentrations, a modern stopped-flow Bio-Logic SFM-300 spectrophotometer instrument was used. This consists of a mechanical sub-system with three machined syringes, one valve block with 3 × 3-way valves, with one or two optional mixers and one aging loop. To allow temperature regulation of the reactant containers, the SFM syringes, valves, delay lines, and cuvettes were enclosed in a water jacket. The syringe plungers of the SFM were driven by stepping motors through ball screws. The speed capability with all syringes running gives a dead-time below 1 ms in the observation cuvette. The spectral curves were recorded with a high-speed Bio-Logic Diode Array DAD MMS-UV/1500–1 spectrophotometer (0.8 ms per spectrum). This procedure drastically reduced the hydrolysis effect at time t = 0, where the spectral curves were recorded.

For indoles **6** to **8**, stable in acidic media, the cell samples were prepared by adding 20 μL of a solution of substrate in pure acetonitrile by syringe into 2 mL of aqueous perchloric acid of the required acidity in each case, the resulting final substrate concentration being 5×10^{-5} M in 1 % acetonitrile/water for all acidities used. The blank of the reference cell was prepared by addition (20 μL) of pure acetonitrile (Biohit Proline Electronic micropipette, inaccuracy 0.5 %, imprecision 0.2 %) into mineral acid of the proper concentration (2 mL). The spectral curves were recorded on a Hewlett–Packard 8453 spectrophotometer fitted with a diode array detection system and a temperature cell holder adapter, electrically regulated and controlled by computer. The absorbance readings were

performed at least in duplicate. The Lambert–Beer law was assessed at the working wavelengths. The acidity functions used were those available for aqueous mineral acid.

Acknowledgments

Financial support by the Junta de Castilla y León (project BU26-02) and the Spanish Ministerio de Ciencia y Tecnología (project BQU2002–01061) is gratefully acknowledged. Thanks are due to Dr. J. Delgado and Dr. R. Sanz (Burgos University) for synthesizing and characterizing one of the compounds.

[1] T. Y. Shen, *J. Am. Chem. Soc.* **1963**, *85*, 488–489.
[2] H. D. von Dell, M. Doersing, W. Fischer, H. Jacobi, R. Kamp, G. Köhler, G. Schöllnhammer; *Arzneim-Forsch./ Drug Res.* **1980**, *30-II-8a*, 1391–1398.
[3] a) H. A. Archontaki, *Analyst* **1995**, *120*, 2627–2634; b) B. R Hajratwala, J. E. Dawson; *J. Pharm. Sci.* **1977**, *66*, 27–29; c) A. Cipiciani, C. Ebert, P. Linda, F. Rubessa, G. Savelli, *J. Pharm. Sci.* **1983**, *72*, 1075–1076; d) J. Arcos, J. Lopez-Palacios, J. M. Leal, P. Sanchez Batanero, F. Mata.; *Bull. Soc. Chim. Fr.* **1991**, *128*, 314–318; e) G. Alibrandi, S. Coppolino, S. D′Aliberti, P. Ficarra, N. Micali, A. Villari, *J. Pharm. Sci.* **2003**, *92(8)*, 1730–1733.
[4] C. B. Xue, M. E. Voss, D. J. Nelson, J. J. W. Duan, R. J. Cherney, I. C. Jacobson, X. He, J. Roderick, L. Chen, R. L. Corbett, L. Wang, D. T. Meyer, K. Kennedy, W. F. De Grado, K. D. Hardman, C. A. Teleha, B. D. Jaffee, R. Q. Liu, R. A. Copeland, M. B. Covington, D. D. Christ, J. M. Trzaskos, R. C. Newton, R. L. Magolda, R. R. Wexler, C. P. Decicco, *J. Med. Chem.* **2001**, *44*, 2636–2660.
[5] A. Bagno, C. Comuzzi, G. Scorrano, *J. Am. Chem. Soc.* **1994**, *116*, 916–924.
[6] a) L. P. Hammett, A. J. Deyrup, *J. Am. Chem. Soc.* **1932**, *54*, 2721–2739; b) L. P. Hammett, *Physical Organic Chemistry. Reaction Rates, Equilibria and Mechanism*, 2nd ed., McGraw–Hill, New York, **1970**.
[7] R. W. Taft, Jr., *J. Am. Chem. Soc.* **1960**, *82*, 2965–2966.
[8] a) A. Mörikofer, W. Simon, E. Heilbronner, *Helv. Chim. Acta* **1959**, *42*, 1737–1749; b) M. J. Jorgenson, D. R. Hatter, *J. Am. Chem. Soc.* **1963**, *85*, 878–883; c) T. G. Bonner, J. C. Lockhart, *J. Chem. Soc.* **1957**, 2840–2841; d) K. Yates, H. Wai, *J. Am. Chem. Soc.* **1964**, *86*, 5408–5413; e) K. Yates, S. A. Shapiro, *Can. J. Chem.* **1972**, *50*, 581–583; f) D. W. Newton, *J. Chem. Educ.* **1982**, *59*, 641–642; g) E. Högfeldt, J. Bigeleisen, *J. Am. Chem. Soc.* **1960**, *82*, 15–20; h) K. N. Bascombe, R. P. Bell, *J. Chem. Soc.* **1959**, 1096–1105; i) T. G. Bonner, J. Phillips, *J. Chem. Soc. B* **1966**, 650–654; j) K. Yates, H. Wai, *Can. J. Chem.* **1965**, *43*, 2131–2133; k) N. C. Deno, P. T. Groves, G. Saines, *J. Am. Chem. Soc.* **1959**, *81*, 5790–5795; l) J. C. D. Brand, W. C. Horning, M. B. Thornley, *J. Chem. Soc.* **1952**, 1374–1383; m) F. H. Westheimer, M. S. Kharasch, *J. Am. Chem. Soc.* **1946**, *68*, 1871–1876; n) A. M. Lowen, M. A. Murray, G. Williams, *J. Chem. Soc.* **1950**, 3318–3322; p) M. A. Murray, G. Williams, *J. Chem. Soc.* **1950**, 3322–3326; q) V. Gold, B. W. Hawes, *J. Chem. Soc.* **1951**, 2102–2111; r) N. C. Deno, H. E. Berkheimer, W. L. Evans, H. J. Peterson, *J. Am. Chem. Soc.* **1959**, *81*, 2344–2347; s) A. J. Kresge, H. J. Chen, Y. Chiang, *J. Chem. Soc. Chem. Commun.* **1972**, 969–970; t) A. J. Kresge, Y. Chiang, *Proc. Chem. Soc. London* **1961**, 81; v) K. Yates, J. C. Riordan., *Can. J. Chem.* **1965**, *43*, 2328–2335; y) D. G. Lee, R. Cameron, *J. Am. Chem. Soc.* **1971**, *93*, 4724–4728; z) E. M. Arnett, R. P. Quirk, J. J. Burke, *J. Am. Chem. Soc.* **1970**, *92*, 1260–1266.
[9] E. M. Arnett, G. W. Mach, *J. Am. Chem. Soc.* **1964**, *86*, 2671–2677.
[10] K. Yates, J. B. Stevens, A. R. Katritzky, *Can. J. Chem.* **1964**, *42*, 1957–1970.
[11] K. Yates, H. Wai, G. Welch, R. A. McClelland, *J. Am. Chem. Soc.* **1973**, *95*, 418–426.
[12] S. A. Attiga, C. H. Rochester, *J. Chem. Soc., Perkin Trans. 2* **1975**, 1411–1414.
[13] a) M. A. Paul, F. A. Long., *Chem. Rev.* **1957**, 57, 1–45; b) N. C. Deno, J. J. Jaruzelski, A. Schriesheim, *J. Am. Chem. Soc.* **1955**, *77*, 3044–3055; c) N. C. Deno, J. J. Jaruzelski, A. Schriesheim, *J. Org. Chem.* **1954**, *19*, 155–167; d) E. M. Arnett, G. W. Mach, *J. Am. Chem. Soc.* **1966**, *88*, 1177–1183; e) A. R. Katritzky, A. J. Waring, K. Yates, *Tetrahedron* **1963**, *19*, 465–469.
[14] a) C. J. O'Connor, *J. Chem. Educ.* **1969**, *46*, 686–689; b) D. Dolman, R. Stewart, *Can. J. Chem.* **1967**, *45*, 903–910, c) P. Vetesník, K. Rothschein, J. Socha, M. Vecera, *Collect. Czech. Chem. Commun.* **1969**, 34, 1087–1093; d) B. Nahlovsky, V. Chvalovsky, *Collect. Czech. Chem. Commun.* **1968**, 33, 3122–3130.
[15] R. A. Cox, K. Yates, *Can. J. Chem.* **1983**, *61*, 2225–2243.
[16] K. Yates, R. A. McClelland, *J. Am. Chem. Soc.* **1967**,*89*, 2686–2692.
[17] W. D. Chandler, D. G. Lee, *Can. J. Chem.* **1990**, *68*, 1757–1761.
[18] a) J. F. Bunnett, F. P. Olsen, *Can. J. Chem.* **1966**, *44*, 1899–1916; b) J. F. Bunnett, F. P. Olsen, *J. Chem. Soc., Chem. Commun.* **1965**, 601–602.
[19] a) N. C. Marziano, G. M. Cimino, R. C. Passerini, *J. Chem. Soc., Perkin Trans. 2* **1973**, 1915–1922; b) N. C. Marziano, P. G. Traverso, R. C. Passerini, *J. Chem. Soc., Perkin Trans. 2* **1977**, 309–313; c) N. C. Marziano, A. Tomasin, P. G. Traverso, A. Tomasin, *J. Chem. Soc., Perkin Trans. 2* **1981**, 1070–1075; d) R. Passerini, N. C. Marziano, P. Traverso, *Gazz. Chim. Ital.* **1975**, *105,* 901–906.
[20] R. A. Cox, K. Yates, *J. Am. Chem. Soc.* **1978**, *100*, 3861–3867.
[21] a) A. Levi, G. Modena, G. Scorrano, *J. Am. Chem. Soc.* **1974**, *96*, 6585–6588; b) P. Bonvicini, A. Levi, V. Lucchini, G. Modena, G. Scorrano, *J. Am. Chem. Soc.* **1973**, *95*, 5960–5964; c) P. De Maria, G. Consiglio, C. Arnone, D. Spinelli, *J. Chem. Soc., Perkin Trans. 2* **1983**, 481–484; d) G. Scorrano, V. Lucchini, *J. Org. Chem.* **1979**, *44*, 1726–1727.
[22] J. T. Edward, S. C. Wong, *J. Am. Chem. Soc.* **1977**, *99*, 4229–4232.
[23] J. W. Barnett, C. J. O'Connor, *Aust. J. Chem.* **1973**, *26*, 2083–2088.
[24] R. A. Cox, *Adv. Phys. Org. Chem.* **2000**, *35*, 1–66.
[25] C. Perrin, *J. Am. Chem. Soc.* **1964**, *86*, 256–258.
[26] a) R. I. Zalewski, *J. Chem. Soc., Perkin Trans. 2* **1979**, 1637–1639; b) Ü. L. Haldna, *Progr. Phys. Org. Chem.* **1990**, *18*, 65–75; c) Ü. L. Haldna, K. Linask, H. Kuura, M. Kudryashova, *Mendeleev Commun.* **1994**, 22–23; d) A. Bagno, V. Lucchini, G. Scorrano, *Bull. Soc. Chim. Fr.* **1987**, 563–572.
[27] B. García, S. Ibeas, F. J. Hoyuelos, J. M. Leal, F. Secco, M. Venturini, *J. Org. Chem.* **2001**, *66*, 7986–7993.
[28] a) B. García, S. Ibeas, J. M. Leal, M. L. Senent, A. Niño, C. Muñoz-Caro, *Chem. Eur. J.* **2000**, *6*, 2644–2652; b) B. García, S. Ibeas, J. M. Leal, *J. Phys. Org. Chem.* **1996**, *9*, 593–597; c) B. García, R. M. Casado, J. Castillo, S. Ibeas, I. Domingo, J. M. Leal, *J. Phys. Org. Chem.* **1993**, *6*, 101–105; d) B. García, J. Arcos, P. L. Domingo, J. M. Leal, *Anal. Lett.* **1991**, *24*, 391–411.
[29] A. Bagno, G. Scorrano, R. A. More O'Ferrall, *Rev. Chem. Interm.* **1987**, *7*, 313–352.
[30] R. A. Cox, K. Yates, *Can. J. Chem.* **1981**, *59*, 2116–2124.
[31] R. A. Cox, K. Yates, *Can. J. Chem.* **1981**, *59*, 1560–1567.
[32] R. Noto, M. Gruttadauria, D. Spinelli, G. Consiglio, *J. Chem. Soc., Perkin Trans. 2* **1990**, 1975–1978.
[33] R. F. Cookson, *Chem. Rev.* **1974**, *74*, 5–28.
[34] L. A. Flexser, L. P. Hammett, A. Dingwall, *J. Am. Chem. Soc.* **1935**, *57*, 2103-2115.
[35] a) G. E. Dunn, F. L. Kung, *Can. J. Chem.* **1966**, *44*, 1261–1270; b) R. I. Zalewski, G. E. Dunn, *Can. J. Chem.* **1969**, *47*, 2263–2270.

[36] C. D. Johnson, A. R. Katritzky, B. J. Ridgewell, N. Shakir, A. M. White, *Tetrahedron* **1965**, *21*, 1055–1059.
[37] C. T. Davis, T. A. Geissman, *J. Am. Chem. Soc.* **1954**, *76*, 3507–3511.
[38] R. Stewart, K. Yates, *J. Am. Chem. Soc.* **1960**, *82*, 4059–4061.
[39] B. S. Andonovski, G. M. Stojkovic, *Acta Chim. Slov.* **2000**, *47*, 349–362.
[40] R. A. Cox, I. Onyido, E. Buncel, *J. Am. Chem. Soc.* **1992**, *114*, 1358–1363.
[41] a) E. Buncel, I. Onyido, *Can. J. Chem.* **1986**, *64*, 2115–2116; b) I. Onyido, L. U. Opara, *J. Chem. Soc., Perkin Trans. 2* **1989**, 1817–1821.
[42] a) J. T. Edward, S. C. Wong, *Can. J. Chem.* **1962**, *40*, 966–975; b) E. M. Arnett, G. Scorrano, *Adv. Phys. Org. Chem.* **1986**, *13*, 83–153; c) A. Bagno, C. Comuzzi, G. Scorrano, *J. Am. Chem. Soc.* **1994**, *116*, 916–924; d) A. Ebber, Ü. L. Haldna, A. Murshak, *Org. React. (Tartu)* **1986**, *23*, 40–50.
[43] R. I. Zalewski, S. Geribaldi, *J. Chem. Soc., Perkin Trans. 2* **1988**, 113–115.
[44] B. García, M. S. Muñoz, S. Ibeas, J. M. Leal, *J. Org. Chem.* **2000**, *65*, 3781–3787.
[45] S. Geribaldi, A. Grec-Luciano, P. C. Maria, M. Azzaro, *J. Chim. Phys., Phys.-Chim. Biol.* **1982**, *79*, 103–114.
[46] a) E. R. Malinowski, D. G. Howery. *Factor Analysis in Chemistry.* Wiley-Interscience, New York, **1980**; b) J. L. Simonds, *J. Opt. Soc. Am.* **1963**, *53*, 968–974.
[47] R. L. Hinman, J. Lang, *J. Am. Chem. Soc.* **1964**, *86*, 3796–3806.
[48] A. J. Kresge, H. J. Chen, G. L. Capen, M. F. Powell, *Can. J. Chem.* **1983**, *61*, 249–256.
[49] D. T. Davies, *Aromatic Heterocyclic Chemistry.* Oxford University Press, **1997**.
[50] R. L. Hinman, E. B. Whipple, *J. Am. Chem. Soc.* **1962**, *84*, 2534–2539.
[51] B. García, J. M. Leal, *J. Phys. Org. Chem.* **1991**, *4*, 413–419.
[52] E. M. Arnett, G. Y. Wu, *J. Am. Chem. Soc.* **1960**, *82*, 5660–5665.
[53] G. Berti, A. Da Settimo, D. Segnini, *Gazz. Chim. Ital.* **1961**, *91*, 571.
[54] a) R. Stewart. *The Proton: Applications to Organic Chemistry.* Academic Press, London, **1985**; b) A. Bagno, G. Scorrano, *J. Phys. Chem.* **1996**, *100*, 1536–1544 and 1545–1553; c) M. Liler, D. Markovic. *J. Chem. Soc., Perkin Trans. 2* **1982**, 551–558.
[55] a) M. Liler, *J. Chem. Soc., Perkin Trans. 2* **1974**, 71–76; b) M. Liler, *J. Chem. Soc., Chem. Commun.* **1972**, 527–528; c) M. Liler, *J. Chem. Soc., Perkin Trans. 2* **1972**, 816–820; d) M. Liler, *J. Chem. Soc. (B)***1971**, 334–338; e) M. Liler, *J. Chem. Soc. B* **1969**, 385–389; f) J. T. Edward, H. S. Chang, K. Yates, R. Stewart, *Can. J. Chem.* **1960**, *38*, 1518–1525.
[56] a) C. L. Perrin, T. J. Dwyer, J. Rebek, R. J. Duff, *J. Am. Chem. Soc.* **1990**, *112*, 3122–3125; b) C. L. Perrin, *Acc. Chem. Res.* **1989**, *22*, 268–275; c) C. L. Perrin, *J. Am. Chem. Soc.* **1974**, *96*, 5628–5631 and 5631–5632.
[57] a) C. L. Perrin, E. R. Johnston, *J. Am. Chem. Soc.* **1979**, *101*, 4753–4754; b) S. F. Waelder, L. Lee, A. G. Redfield, *J. Am. Chem. Soc.* **1975**, *97*, 2927–2928; c) A. G. Redfield, S. Waelder, *J. Am. Chem. Soc.* **1979**, *101*, 6151–6162.
[58] a) P. De María, C. L. Barbieri, D. Spinelli, C. Dell'Erba, M. Novi, G. Petrillo, F. Sancassan, *J. Chem. Soc., Perkin Trans. 2* **1991**, 373–376; b) G. Alberghina, M. E. Amato, S. Fisichella, S. Occhipinti, M. Liler, *J. Chem. Soc., Perkin Trans. 2* **1980**, 1721–1724.
[59] M. Liler, *Adv. Phys. Org. Chem.* **1975**, *11*, 267–392.
[60] a) G. Fraenkel, C. Franconi, *J. Am. Chem. Soc.* **1960**, *82*, 4478–4483; b) A. Berger, A. Loewenstein, S. Meiboom, *J. Am. Chem. Soc.* **1959**, *81*, 62–67; c) R. J. Gillespie, T. Birchall, *Can. J. Chem.* **1963**, *41*, 148–155; d) C. L. Perrin, E. R. Johnston, *J. Am. Chem. Soc.* **1979**, *101*, 4753–4754; e) S. F. Waelder, L. Lee, A. G. Redfield, *J. Am. Chem. Soc.* **1975**, *97*, 2927–2928; f) A. G. Redfield, S. Waelder, *J. Am. Chem. Soc.* **1979**, *101*, 6151–6162.
[61] a) O. Núñez, J. Rodriguez, L. Angulo, *J. Phys. Org. Chem.* **1993**, *7*, 80–89; b) A. J. Kresge, *Acc. Chem. Res.* **1975**, *8*, 354–360.
[62] a) P. Proctor, N. P. Gensmantel, M. I. Page, M. Liler, *J. Chem. Soc., Perkin Trans. 2***1982**, 1185–1192; b) A. R. Fersht, *J. Am. Chem. Soc.* **1971**, *93*, 3504–3515.
[63] F. J. Hoyuelos, PhD Thesis, Universidad de Burgos, Spain, **2003**.
[64] R. A. Cox, R. Stewart, *J. Am. Chem. Soc.* **1976**, *98*, 488–494.
[65] V. Lucchini, G. Modena, G. Scorrano, R. A. Cox, K. Yates, *J. Am. Chem. Soc.* **1982**, *104*, 1958–1959.
[66] R. A. Cox, K. Yates, *Can. J. Chem.* **1984**, *62*, 2155–2160.
[67] a) M. Lajunen, J. M. Koskinen, *Acta Chem. Scand.* **1994**, *48*, 788–791; b) M. Lajunen, E. Kaitaranta, M. Dahlqvist, *Acta Chem. Scand.* **1994**, *48*, 399–403.
[68] R. J. Sundberg, *Indoles*, Academic Press, **1996**.

Received: June 21, 2004

