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Acid-base Properties of Terbutaline in Terms of Protonation Macro- and Microconstants

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Abstract

The acid/base chemistry of terbutaline was characterized at the molecular level in terms of protonation macroconstants and microconstants. The macroconstants were measured by potentiometry and calculated by standard evaluation methods.

The stepwise macroconstant values were $\log K_1 = 11{\cdot}01$, $\log K_2 = 9{\cdot}89$, and $\log K_3 = 8{\cdot}57$ at 25·0°C and 0·2 M ionic strength. The microconstants were deduced using the relationships between macro- and microconstants and an appropriate data set of model compounds (resorcinol and phenylephrine). The molecule of terbutaline contains three ionizable functional groups. In the unprotonated form of the molecule, the two identical phenolate groups are slightly more basic than the secondary amino group, whereas the amino basicity significantly exceeds that of the phenolate site, when the other phenol is protonated. This is due to the large phenolate–phenolate intramolecular interaction. The phenolate–phenolate and the phenolate–amino interactivity parameters were found to be −1·21 and −0·41 log E units, respectively.

Terbutaline is a selective β_2-adrenoceptor stimulant, widely used in the treatment of bronchial asthma. In an effort to find relationships between the biological and chemical properties, several characteristics of the molecule have been studied. The pharmacology and pharmacokinetics of terbutaline were reported by Wetterlin (1972) and Borgström et al (1989), respectively. Its analytical determination has been carried out by various methods, such as ion-pair extraction (Modin & Johansson 1971), HPLC in biological samples (Sagar et al 1992), a number of capillary electrophoresis techniques (Ackermans et al 1992) and coulometric assay in pharmaceutical dosage forms (Nikolic et al 1993). The ionization and protonation properties were reported by Ahuja & Ashman (1990) in terms of pK_a values (8·8, 10·1, 11·2), with no indication of the experimental method and no assignment of basicities to moieties. The ionization and distribution equilibria in isopropanol/water were quantitated by Liu et al (1992), where the molecule was treated as bifunctional; thus the third proton-binding process was ignored.

The ionization (protonation, acid/base) equilibria are of fundamental importance for any further binding studies. It is generally accepted that biological reactions (membrane penetration, receptor-binding, enzymatic decomposition) utilize different protonation/ionization forms of the molecule. Also, the understanding of kinetics and thermodynamics of metal complexation and drug metabolism necessitate the determination of protonation constants. Interactions with the ubiquitous proton are therefore a precondition to obtain an insight into chemical and biological processes at the molecular level.

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As a part of our studies on the medicinal chemistry of amphoteric drugs (Takács-Novák et al 1995), we investigated the acid/base properties of terbutaline. Here we characterize the terbutaline basicity for the molecule as a whole, and for its functional groups.

Materials and Methods

Terbutaline sulphate was generously supplied by Egis Pharmaceutical Works (Budapest) and used without further purification. Resorcinol was of pharmacopoeial grade (Ph. Hung. VII) and all other reagents were of analytical grade.

The protonation macroconstants were measured by potentiometry at 25·0 ± 0·1°C under N_2 and at constant ionic strength (I = 0·2 M, auxiliary electrolyte: KCl). A 4×10^{-2}M solution of examined compounds was titrated with 1 M KOH. The apparatus, electrode calibration process and the calculation of complex products were described earlier (Takács-Novák et al 1990).

The pH-dependence of the UV spectra of terbutaline was studied using a conventional method (Albert & Serjeant 1971). Two samples of 0·1 mM terbutaline solutions were prepared in either 0·1 M HCl or 0·1 M NaOH, with a total ionic strength of 0·2 M. By mixing the acidic and basic stock solutions, ten solutions in the pH range 7·5–12·0 were obtained and their spectra were recorded on a Hewlett-Packard 8452A diode-array spectrometer.

Results and Discussion

Terbutaline, in its most basic form, contains three functional groups: two phenolate and one secondary amino group. The three functional groups are of similar basicity and associate with protons in overlapping processes. The widely used

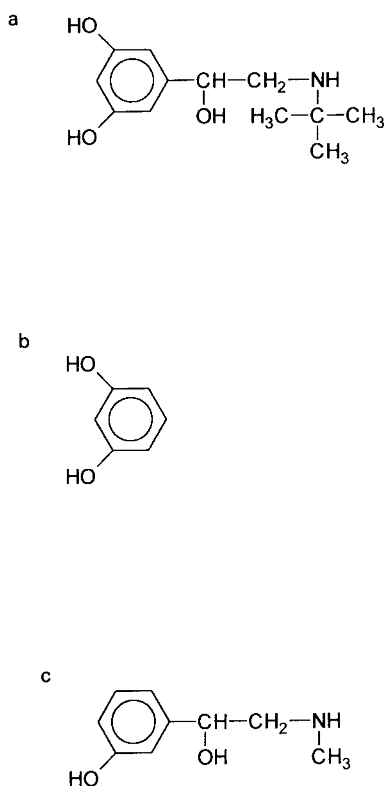

FIG. 1. Chemical structure of terbutaline (a) and the model compounds resorcinol (b) and phenylephrine (c).

macroscopic protonation constants characterize the acid/base properties of the molecule as a whole, but can not be assigned to individual (specific) binding sites. This is especially true for overlapping equilibria (Noszál 1990). In such cases basicities of the individual binding sites can be correctly characterized in terms of protonation microconstants.

The determination of microconstants always includes two or more techniques. An essential technique is pH-metry, which provides information on the total proton-binding of the molecule. The inevitable pH-metry is supplemented tentatively with a spectroscopic method (UV-vis, NMR), which selectively monitors protonation of one or more of the groups. When selective monitoring is hampered by a large number or close proximity or high similarity of the groups, then deductive methods must be applied.

In terbutaline, the close proximity of the proton-binding sites precludes the specific, unambiguous assignment of spectroscopic signals to any group of definite identity.

We therefore used two compounds as models of terbutaline sub-units. These compounds (resorcinol and phenylephrine; Fig. 1) contain a reduced number of functional groups, and their appropriate interactivity parameters can be introduced into the microspeciation scheme. Hence, all the microconstants of terbutaline could be deduced. Our deductively obtained microconstants were augmented by a spectrophotometric assay, which provided information on the pH-dependent protonation state of the phenolate groups and confirmed the microconstant values.

Fig. 2 shows the microscopic protonation scheme of terbutaline. In general, the microspeciation scheme of a trivalent base consists of 8 microspecies and 12 micro-

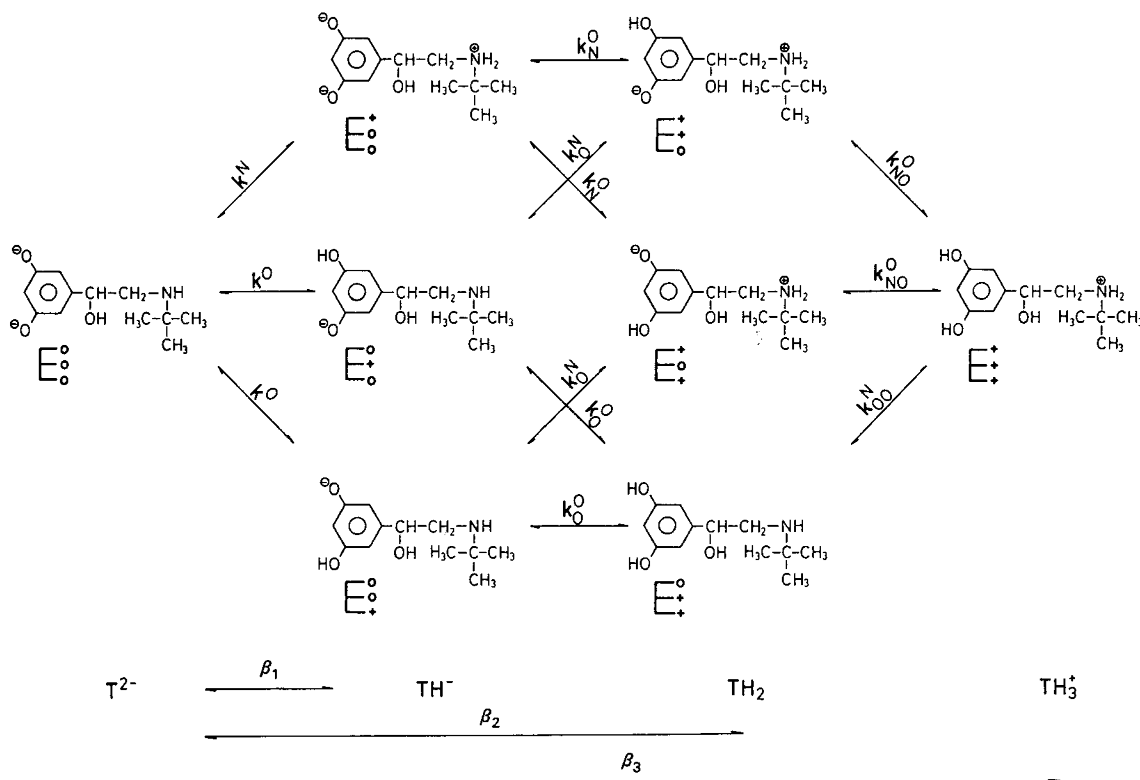

FIG. 2. Protonation scheme for terbutaline.

Table 1. Complex products and protonation macroconstants of terbutaline.

Complex products	Stepwise macroconstants
$\log \beta_1 = 11{\cdot}01$	$\log K_1 = 11{\cdot}01 \pm 0{\cdot}07$
$\log \beta_2 = 20{\cdot}90$	$\log K_2 = 9{\cdot}89 \pm 0{\cdot}04$
$\log \beta_3 = 29{\cdot}47$	$\log K_3 = 8{\cdot}57 \pm 0{\cdot}02$

constants. The two identical phenolate groups and the inherent symmetry in terbutaline implies only 6 nonidentical microspecies and 7 different microconstants. Accordingly, the relationships between β_1, β_2, β_3 macroconstants, T^{2-}, TH^-, TH_2 and TH_3^+ macrospecies concentrations and the $\overset{O}{E}{}^{O}_{O}$, $\overset{+}{E}{}^{O}_{O}$..., $\overset{+}{E}{}^{+}_{+}$ microspecies concentrations are as follows:

$$\beta_1 = \frac{[TH^-]}{[T^{2-}][H^+]} = \frac{[\overset{+}{E}{}^{O}_{O}] + [\overset{O}{E}{}^{+}_{O}] + [\overset{O}{E}{}^{O}_{+}]}{[\overset{O}{E}{}^{O}_{O}][H^+]} = 2k^O + k^N \quad (1)$$

$$\beta_2 = \frac{[TH_2]}{[T^{2-}][H^+]^2} = \frac{[\overset{+}{E}{}^{+}_{O}] + [\overset{+}{E}{}^{O}_{+}] + [\overset{O}{E}{}^{+}_{+}]}{[\overset{O}{E}{}^{O}_{O}][H^+]^2} = 2k^N k^O_N + k^O k^O_O \quad (2)$$

$$\beta_3 = \frac{[TH_3^+]}{[T^{2-}][H^+]^3} = \frac{[\overset{+}{E}{}^{+}_{+}]}{[\overset{O}{E}{}^{O}_{O}][H^+]^3} = k^N k^O_N k^O_{N-O} \quad (3)$$

where N and O are the amino and phenolate group, respectively. The superscript of the k microconstants denotes the group protonating in the process in question, and the subscript (if any) denotes the group protonated.

The macroconstants can be determined from pH-metric data by standard methods. The protonation macroconstants of terbutaline represented as cumulative constants (complex products) and stepwise, concentration constants are listed in Table 1 in log units. The standard deviation of five parallel potentiometric determinations is also indicated. The greatest uncertainty ($\pm$ 0·07) is with the log K values (log β_1), due to the limited accuracy of pH measurement at high pH values.

Equations 1–3 show, however, that at least two additional pieces of information are needed to elucidate the microconstants.

It has recently been recognized (Noszál & Sándor 1989) that interactivity parameters of the same moiety retain their values in different molecules. Thus, interactivity parameters determined for model compounds can be introduced into complicated microspeciation systems, and an appropriate calculation procedure may result in the determination of all microconstants.

For the protonation processes of terbutaline, two inter-moiety interactions exist: the phenolate–phenolate and the amino–phenolate interactions, with E_{O-O} and E_{N-O} interactivity parameters, respectively. The interactivity parameter is a measure of the reciprocal decrease in basicity at

Table 2. Protonation macro- and microconstants of resorcinol.

Stepwise macroconstants	Microconstants
$\log K_1 = 11{\cdot}09 \pm 0{\cdot}01$	$\log k^O = 10{\cdot}79$
$\log K_2 = 9{\cdot}28 \pm 0{\cdot}01$	$\log k^O_O = 9{\cdot}58$

Table 3. Protonation microconstants of terbutaline.

Microconstants		
$\log k^N = 10{\cdot}36$	$\log k^N_O = 9{\cdot}35$	$\log k^N_{O\text{-}O} = 9{\cdot}54$
$\log k^O = 10{\cdot}60$	$\log k^O_N = 10{\cdot}19$	$\log k^O_{N\text{-}O} = 8{\cdot}98$
	$\log k^O_O = 9{\cdot}39$	

one site upon protonation of the other site. The relationships between microconstants and interactivity parameters are given in equations 4 and 5:

$$E_{O-O} = k^O_O/k^O = k^O_{N-O}/k^O_N \quad (4)$$

$$E_{N-O} = k^O_N/k^O = k^N_O/k^N = k^N_{O-O}/k^N_O = k^O_{N-O}/k^O_O \quad (5)$$

The E_{O-O} phenolate–phenolate interactivity parameter can be calculated from the data for resorcinol, which is the evident aromatic sub-unit of terbutaline.

Using consistent notations, the following relationships hold for protonation of the doubly-charged resorcinate anion:

$$\beta_1 = 2k^O \quad (6)$$

$$\beta_2 = k^O k^O_O = k^O k^O E_{O-O} \quad (7)$$

The symmetry of the resorcinol allows the determination of not only the macro-, but also the microconstants; the values shown in Table 2 are in good agreement with literature data: 11·06, 9·30 (Martell & Smith 1982). From these data the interactivity parameter, log E_{O-O}, was calculated as $-1{\cdot}21$. The amino–phenolate log E_{N-O} value of $-0{\cdot}41$ was obtained from the work of Quintero et al (1989).

Combining equations 1–5, yields:

$$\beta_1 = 2k^O + k^N \quad (8)$$

$$\beta_2 = 2(k^N k^O E_{N-O}) + (k^O)^2 E_{O-O} \quad (9)$$

$$\beta_3 = k^N (k^O)^2 E_{O-O} (E_{N-O})^2 \quad (10)$$

Since β_1, β_2, β_3 values are experimental data, and E_{O-O} and E_{N-O} are obtained as described above, the 8–10 system of equations is redundant. The calculated microconstant values are listed in Table 3.

We also performed a pH-metric-photometric study on terbutaline. In order to follow the phenolate protonation equilibria selectively, we recorded UV spectra in the neutral-basic range, between 240 and 320 nm. Such a combination of pH-metric and photometric assay has been used to elucidate microconstants for phenolate–amino binding site-containing systems (Martin 1971).

For terbutaline, spectra at pH $\leqslant$ 10·5 show a definite isosbestic point near 280 nm, indicating that below pH 10·5, virtually two spectrum-influencing species exist only: the mono- and diprotonated phenolate-containing ones, for the protonation state of the amino group does not influence the spectrum at this wavelength (Fig. 3). The spectral changes at pH as low as 7·5 indicate, however, that the amino and initial phenolate protonations significantly decrease the second phenolate basicity, in accordance with the log k^O_{N-O} value of 8·98. On the other hand, spectra above pH 10·5 have a different character, evidence for deprotonation of the second phenolate hydroxyl group.

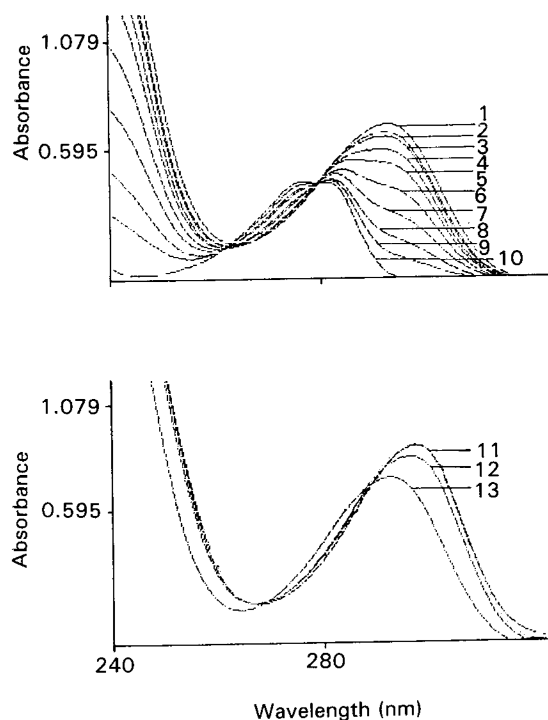

FIG. 3. UV spectra of terbutaline as a function of pH: 1, pH = 10·5; 2, pH = 9·8; 3, pH = 9·5; 4, pH = 9·2; 5, pH = 8·9; 6, pH = 8·6; 7, pH = 8·3; 8, pH = 8·0; 9, pH = 7·5; 10, 0·01 M HCl; 11, 0·1 M NaOH; 12, pH = 12·0; 13, pH = 10·5.

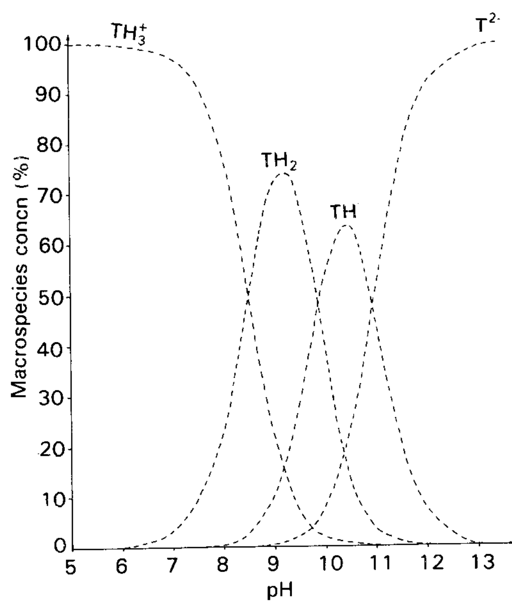

FIG. 4. Distribution diagram of macrospecies.

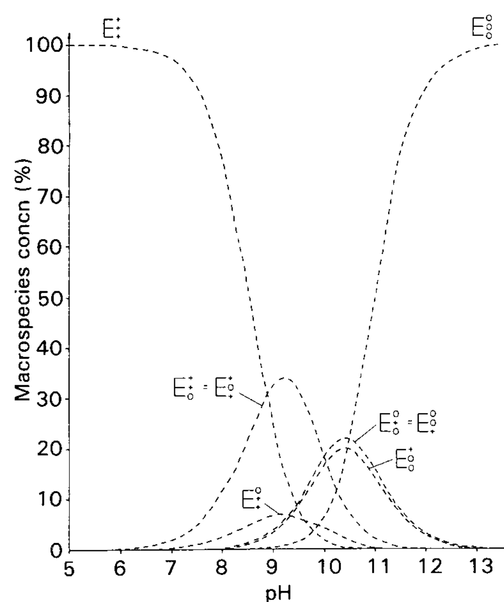

FIG. 5. Distribution diagram of microspecies.

The lack of individual molar absorption coefficients for all the spectrum-influencing species did not allow the determination of the pH-metric-photometric microconstant. Nevertheless, this spectroscopic assay has qualitatively supported the deductively obtained microconstants. It can be seen from the data in Table 3 that the phenolate basicities are slightly above that of the amino, when none of the other binding-sites are protonated (log k^O = 10·60, log k^N = 10·36) and when the other type of site is protonated (log k^O_N = 10·19, log k^N_O = 9·95). However, the amino basicity significantly exceeds that of the phenolate when the other phenolate is protonated (log k^N_O = 9·95, log k^O_O = 9·39, and log $k^N_{O\text{-}O}$ = 9·54, log $k^O_{N\text{-}O}$ = 8·98).

With regard to the fate of terbutaline in-vivo the above order of the microconstants has a special significance. It is generally accepted that phenolate protonation constants significantly increase in media of lower relative permittivity, whereas amino basicity changes are much less significant under similar circumstances. These properties provide terbutaline with a high degree of ionization, charge-distribution and electron-density versatility in different biological media of various dielectric constants, such as intra- and intercellular liquids, membrane and receptor surfaces.

Our aqueous protonation constants could be used to construct distribution diagrams. The pH-dependent relative macrospecies concentrations are shown in Fig. 4, and the analogous microspecies distributions are shown in Fig. 5. The isoelectric point (where the molecule bears a zero overall charge) is at pH 9·82.

Table 4. Relative concentrations (%) of microspecies in intestine, blood and ileum.

pH	$E_{\substack{o\\o\\o}}$	$E_{\substack{+\\o\\o}}$	$E_{\substack{o\\+\\o}} = E_{\substack{o\\o\\+}}$	$E_{\substack{+\\+\\o}} = E_{\substack{+\\o\\+}}$	$E_{\substack{o\\+\\+}}$	$E_{\substack{+\\+\\+}}$
5·30	2·69 × 10^{-12}	3·09 × 10^{-7}	5·37 × 10^{-7}	2·39 × 10^{-2}	6·60 × 10^{-3}	99·95
7·40	5·02 × 10^{-6}	4·59 × 10^{-3}	7·97 × 10^{-3}	2·83	7·79 × 10^{-1}	93·65
8·00	2·66 × 10^{-4}	6·10 × 10^{-2}	1·05 × 10^{-1}	9·45	2·60	78·57

At pH 1·5 (the pH of gastric fluid), terbutaline exists overwhelmingly in the protonated, cationic form, where concentrations of all other microspecies are negligible.

Table 4 shows the relative (%) concentration for all microspecies at three physiologically important pH values (intestine, blood, ileum).

Assuming that terbutaline is absorbed by passive diffusion in the non-ionized form, these data explain its low (7–20%) bioavailability after oral administration (Ahuja & Ashman 1990).

As a further utilization of the microspeciation data, we studied the pH-dependent lipophilicity of terbutaline by determining the apparent octanol/water partition coefficients. The true partition coefficient, referring to partition of the neutral microspecies, was calculated using protonation microconstants (Takács-Novák et al 1995).

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