Table IV—Local Anesthetic Activity of Benzo[*b*]furan Derivatives[a]

Compound[b]	Concentration, %	Potency	Duration, min
IIIa	1	0.85 (0.75–0.95)	13–18
IIIb	1	1.0	Over 18
	0.5	0.96 (0.91–1.0	16–18
	0.25	0.41 (0.30–0.53)	6–8
IIIc	1	0.75 (0.65–0.85)	10–15
IIId	1	0.85 (0.76–0.93)	13–17
IIIe	1	0.62 (0.51–0.74)	9–15
IIIf	1	1.0	Over 18
	0.5	0.68 (0.57–0.79)	11–13
IIIg	1	0.82 (0.73–0.91)	12–18
IIIh	1	0	0
Vb	1	0.72 (0.61–0.83)	13–15
Ve	1	1.0	Over 18
	0.25	0.75 (0.64–0.85)	11–15
Vf	1	0.97 (0.93–1.0)	17–18
VIIa	1	0	0
VIIb	1	0.96 (0.91–1.0)	16–18
	0.50	0.84 (0.75–0.93)	14–16
	0.25	0.57 (0.45–0.68)	10–14
VIIc	1	0.84 (0.76–0.92)	13–16
VIId	1	0	0
VIII	1	0	0
Cocaine	0.25	1.0	Over 18
	0.125	0.89 (0.82–0.96)	11–18

[a] Surface anesthesia was tested according to the method of M. R. A. Chance and H. J. Lobstein, *J. Pharmacol. Exp. Ther.*, **82**, 203 (1944). Anesthetic potency was calculated for the first 18 min [A. H. Campbell, J. A. Strasse, G. H. Lord, and J. E. Willson, *J. Pharm. Sci.*, **57**, 2045 (1968)]. A potency of 1.00 indicates an onset of anesthesia in 1 min and a duration of at least 18 min. [b] Compounds of Tables I–III not included in this table could not be dissolved in water and were not tested.

EXPERIMENTAL[1]

Compound II—A mixture of I (17.68 g, 0.01 mole) (5) in 100 ml of thionyl chloride was refluxed for 4 hr. The excess of thionyl chloride was evaporated under reduced pressure, and the residue was distilled to give II (16 g, 82%), bp 114–116°/6 mm Hg, mp 64–65° (hexane).

Anal.—Calc. for $C_{10}H_7ClO_2$: C, 61.70, H, 3.60. Found: C, 61.85; H, 3.79.

2-Dimethylaminoethyl 3-Methyl-2-benzo[*b*]furancarboxylate (IIIa)—A solution of 2-dimethylaminoethanol (0.89 g, 0.01 mole) and II (1.945 g, 0.01 mole) in 20 ml of benzene was refluxed for 4 hr. The solvent was evaporated, and the residue was crystallized from ethanol–ethyl acetate to give IIIa (2.55 g, 90%), mp 189–190°; IR (potassium bromide): 1715 cm^{-1} (ester); NMR (deuterochloroform, as free base): δ 7.66–7.17 (m, 4H, aromatic), 4.43 (t, 2H, OCH_2), 2.66 (t, 2H, CH_2N), 2.53 (s, 3H, CH_3), and 2.33 (s, 6H, NCH_3) ppm; *m/e* 247.

Compounds IIIb–IIIh were prepared similarly (Table I).

2-Dimethylaminoethyl 3-Methyl-2-benzo[*b*]furancarbamate (Va)—A solution of IV (2.01 g, 0.01 mole) (2) and 2-dimethylaminoethanol (0.89 g, 0.01 mole) in 20 ml of benzene was refluxed for 4 hr. The solvent was evaporated, and the residue was crystallized as a picrate, mp 134–135°; IR (potassium bromide, as free base): 1720 and 1249 cm^{-1} (ester); NMR (deuterochloroform, as free base): δ 7.50–7.00 (m, 5H, aromatic and NH), 4.23 (t, 2H, OCH_2), 2.56 (t, 2H, CH_2N), 2.23 (s, 6H, NCH_3), and 2.07 (s, 3H, CH_3) ppm; *m/e* 262.

Compounds Vb–Vg were prepared similarly (Table II).

***N*-(2-Diethylaminoethyl)-3-methyl- 2- benzo[*b*]furancarboxamide (VIIb)**—*Method A*—A solution of IV (2.01 g, 0.01 mole) and 2-diethylaminoethylamine (1.16 g, 0.01 mole) in 30 ml of benzene was refluxed for 2 hr, and the solvent was evaporated. The residue was crystallized from petroleum ether to give VIIb (2.5 g, 91%), mp 45–46°; IR (potassium bromide): 3300 (NH) and 1650 (amide) cm^{-1}; NMR (deuterochloroform): δ 7.73–7.00 (m, 5H, aromatic and NH), 3.5 (unresolved t, $CONCH_2$), 2.69 (t, 2H, CH_2N), 2.66 (s, 3H, CH_3), 2.54 (q, 4H, NCH_2), and 1.60 (t, 6H, CH_3) ppm; *m/e* 274.

Method B—A solution of II (1.945 g, 0.01 mole) and 2-diethylaminoethylamine (1.16 g, 0.01 mole) in 30 ml of benzene was refluxed for 2 hr. The solvent was evaporated, and the residue was crystallized from ethyl acetate to give VIIb as the hydrochloride (2.8 g, 90%), mp 129–130°, and as a free base, mp 45–46° (petroleum ether).

Compounds VIIa, VIIc, VIId, and VIII were prepared similarly (Table III).

[1] Melting points were taken on a Kofler hot-stage microscope and are uncorrected. IR spectra were recorded using a Perkin-Elmer 267 spectrophotometer. Mass spectra were recorded on a Varian Mat III instrument. NMR spectra were determined with a Varian T-60A instrument.

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Determination of Ionization Constants in Mixed Aqueous Solvents of Varying Composition by a Single Titration

JEF PEETERS

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Abstract □ A potentiometric titration method is proposed in which only one titration is necessary to obtain pK'a values for different solvent compositions. The method allows the results to be extrapolated to the value for pure water. Examples are given, and the advantages and disadvantages of the method are discussed.

Keyphrases □ Ionization constants—potentiometric determination in mixed aqueous solvents of various composition □ Potentiometry—determination of ionization constants in mixed aqueous solvents of various composition

The use of mixed aqueous solvents for the potentiometric determination of ionization constants has disadvantages and should be avoided if possible. However, the solubility requirements for aqueous titrations are often too demanding, and many compounds are not suited for spectrophotometric analysis. Although Albert and Serjeant

Table I—Titration Data for 1-Methyl-1*H*-imidazole [a]

NaOH Added, ml	pH	Methanol, %	pK'a Value
1.6	5.47	60.34	6.455
2.0	5.71	58.33	6.480
2.4	5.90	56.45	6.512
2.8	6.05	54.69	6.532
3.2	6.19	53.03	6.560
3.6	6.31	51.47	6.578
4.0	6.42	50.00	6.593
4.4	6.53	48.61	6.612
4.8	6.64	47.30	6.632
5.2	6.74	46.05	6.643
5.6	6.85	44.87	6.661
6.0	6.96	43.75	6.675
6.4	7.08	42.68	6.691
6.8	7.21	41.67	6.706
7.2	7.35	40.70	6.713
7.6	7.52	39.77	6.717
8.0	7.76	38.89	6.726

[a] Product (6.5 mg) is converted to its acidic form with 0.85 ml of 0.1 *N* HCl. Then 7 ml of methanol is added and brought to 10 ml with water. The solution is titrated with aqueous 0.01 *N* NaOH. Number of points $n = 17$, regression coefficient $r = -0.9985$, slope $m = -0.013$, intercept $b = 7.235$, F value = 5152, and variance of intercept $s^2 = 7.551 \times 10^{-5}$.

(1) condemned the results obtained in mixed aqueous solvents as being of doubtful value, the method is rather popular, especially for the comparison of closely related compounds.

In a recent review, Cookson (2) discussed the advantages and disadvantages of mixed solvents. The purpose of this note is not to justify the method nor to condemn it; many workers have no alternative and prefer to obtain less accurate results rather than to get entangled in experimental difficulties and time-consuming methods (3–5).

This paper presents a relatively simple titration procedure in which only one titration is necessary to obtain pK'a[1] values for different solvent compositions. Consequently, the method allows the results to be extrapolated to the value for pure water. The method has been applied routinely in this laboratory for about 3 years.

In the conventional method, solutions with different solvent composition are titrated, and the results are extrapolated, mostly by graphical techniques. This method is rather time consuming, since at least three solutions with different solvent composition are titrated with three different titer solutions having compositions equal to the titrated solution. In the proposed method, this step can be avoided.

THEORETICAL

If a compound dissolved in a binary mixture, with a stated ratio in volumes of both constituent solvents, is titrated with a titer solution having another, also stated, ratio in volumes of both constituent solvents, the composition of the resulting solution is continuously changing during the titration. The exact composition can be calculated at each step of the titration:

$$P = \frac{(P_i{}^p V_p) + (P_i{}^t V_t)}{V_p + V_t} \qquad \text{(Eq. 1)}$$

where P is the percent of nonaqueous solvent; $P_i{}^p$ and $P_i{}^t$ are the initial percentages of nonaqueous solvent in the solution and the titrant, respectively; and V_p and V_t are the volumes of the solution and the titrant, respectively. Since, on the other hand, the pK'a can be calculated by well-known formulas (1), an extrapolation of pK'a to the value for pure water can be done with as many points as necessary, provided the titration curve (pH *versus* milliliters of titrant) is available.

During the titration of a compound, *e.g.*, BH^+, with a strong base, the ratio of concentration of BH^+ and B is expressed as:

$$\frac{[BH^+]}{[B]} = \frac{EP}{V_t} - 1 \qquad \text{(Eq. 2)}$$

where EP is the equivalence point indicating the end of the titration.

Substitution of Eq. 2 in the well-known formulas (1) allows calculation of the pK'a at any point of a titration curve and, in combination with Eq. 1, the necessary data pairs are obtained for extrapolation.

Slightly differing expressions are obtained when a base is converted into its acidic form by addition of an excess strong acid prior to titration:

$$P = \frac{(P_i{}^p V_p) + (P_i{}^t V_t) + (P_i{}^a V_a)}{V_p + V_t + V_a} \qquad \text{(Eq. 3)}$$

$$\frac{[BH^+]}{[B]} = \frac{EP_2 - EP_1}{V_t - EP_1} - 1 \qquad \text{(Eq. 4)}$$

where index a indicates the added acid solution; EP_1 is the first equivalence point, indicating the end of the neutralization of the excess acid; and EP_2 is the second equivalence point, indicating the end of the titration.

EXPERIMENTAL

Materials—The following materials were used without further purification: 1-methyl-1*H*-imidazole[2], cinnarizine[3], seperidol hydrochloride[4], diphenoxylate hydrochloride[5], etomidate sulfate[6], and miconazole nitrate[7]. The solvents used were analytical grade.

Water was distilled and deionized by being passed through an intimate mixture of acidic cation- and basic anion-exchange resins[8] before use. Sodium hydroxide and hydrochloric acid solutions were prepared by dilution of concentrated reagents, commercially available[9].

Equipment—Titration curves were recorded with an automatic titration setup[10], equipped with glass and calomel electrodes[11]. The electrode system was standardized with the standard buffer[12] 0.025 *M* KH_2PO_4–0.025 *M* Na_2HPO_4.

Method—If the compound is available as a salt, an appropriate amount is dissolved in the organic solvent and water is added to obtain the desired mixture. If the compound is a free base, the appropriate amount is converted to its acidic form by addition of a small excess of 0.1 *N* HCl; then the organic solvent and water are added.

The solutions are thermostated in a water-jacketed vessel, and 0.01 *N* NaOH is added from a 25-ml buret. After the titration, the pH and the corresponding milliliters of titrant are read from the recorded titration curve. The calculations are performed on a small desk calculator.

RESULTS AND DISCUSSION

Table I illustrates the use of the method for 1-methyl-1*H*-imidazole. This compound was chosen for comparison purposes. The first and the last points of the titration curve were not used for the calculation because equilibrium conditions were not reached at these stages of the titration with the automatic equipment.

The pK'a value in pure water, obtained after extrapolation of the data, was 7.24; 6.95 and 7.20 are given in Ref. 6. A determination using the more conventional technique and extrapolation of data obtained in separate single titrations with 5, 10, 20, 30, 40, and 50% methanol yielded the value of 7.16.

A collection of data obtained with the proposed method and comparison with values determined by other methods are given in Table II. Etomidate is included in the table to demonstrate that very weak bases

[1] The symbol pK'a indicates that the ionization constants are uncorrected values (concentration constants).

[2] Ninety-nine percent, Aldrich-Europe.

[3] USAN for (*E*)-1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)piperazine; Janssen Pharmaceutica, Beerse, Belgium.

[4] USAN for 4-[4-[4-chloro-3-(trifluoromethyl)phenyl]-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone hydrochloride; Janssen Pharmaceutica, Beerse, Belgium.

[5] USP and RINN for ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenyl-4-piperidinecarboxylate monohydrochloride; Janssen Pharmaceutica, Beerse, Belgium.

[6] USAN for (*R*)-(+)-ethyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate; Janssen Pharmaceutica, Beerse, Belgium.

[7] USAN for 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-1*H*-imidazole mononitrate; Janssen Pharmaceutica, Beerse, Belgium.

[8] Elgacan B 114, The Elga Group, England.

[9] Dilut-It, Baker Chemicals, Deventer, Holland.

[10] Radiometer titrator TTT 2, autoburet ABU 12 b, and titrigraph REA 300.

[11] Radiometer glass electrode G 202 C and calomel electrode K 401.

[12] NBS.

Table II—pK'a Values Determined by the Proposed Method and Comparison with Values Determined by Other Methods

Compound	Proposed Method	Comparison Value
Seperidol	8.43	8.44[a]
Cinnarizine	7.47	7.60[a]
Diphenoxylate	7.07	—
Etomidate	Not applicable	4.24[b]
Miconazole	6.91	6.65[a]

[a] Determined by conventional titration method and extrapolation to pure water. [b] Determined by potentiometric titration and UV spectrophotometry.

(pKa < 5) are not suited for the method. Indeed, the addition of methanol to the solution reduced the pH of the medium so that it fell outside the measurable range.

The method yields results comparable with results from the conventional method of performing various titrations in differently composed mixtures and extrapolating to pure water. Moreover, the method is fast and saves a great deal of manipulation time.

In dealing with the determination of pK'a values for insoluble compounds, one commonly uses a binary solvent mixture with a fixed ratio, *e.g.*, methanol–water (1:1), and compares the dissociation constant in that medium. This approach is reasonable as long as the compounds are structurally similar, differing only by some substituents or substituent position. However, when the pKa values of compounds with different structures are to be compared, as in structure–activity relationship studies, not all of the compounds may be soluble in the chosen binary mixture. Furthermore, the influence of the solvent will not be the same on each compound. With the proposed method, the influence of the solvent is extrapolated at least to some extent.

From a fundamental point of view, the method has serious shortcomings: (*a*) linearity over a small composition range does not necessarily justify extrapolation over a considerable distance, (*b*) a single titration does not permit estimation of the precision of individual points on the curve, and (*c*) the method only works for compounds in a narrow pK'a range. In spite of these shortcomings, the method is useful in situations where only approximate pK'a values are necessary for many water-insoluble compounds and where a comparison is to be made between closely related compounds. In comparison with the conventional titration procedure, the proposed method is rapid, easily applicable, and yields good results. Moreover, it is possible to delay the precipitation of a free base during titration by choosing the experimental conditions in such a way that the percentage of organic cosolvent increases together with the concentration of free base.

The method should also be of interest to people studying the influence of mixtures of water and organic solvent on the dissociation behavior of weak acids and weak bases. Indeed, a range of different percentages of the cosolvent is covered by a single titration; consequently, the amount of experimental manipulation could be greatly reduced.

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l-Bunolol Metabolism in Rats: Identification of Urinary Metabolites

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Abstract □ Urine collected for 24 hr from rats given a single oral dose of ^{3}H-*l*-bunolol (10 mg/kg) was found to contain only 25.8% of the dose and more than 30 labeled compounds. Nine compounds were identified and quantified as follows: bunolol (0.35% of urinary tritium), bunolol glucuronide (5.12%), bunolol sulfate (0.08%), dihydrobunolol (0.08%), dihydrobunolol glucuronide (0.74%), dihydrobunolol sulfate (0.12%), hydroxydihydrobunolol (0.58%), β-(5-oxytetralonyl)lactic acid (0.74%), and (5-oxytetralonyl)acetic acid glucuronide (1.12%). The total quantity of identified labeled compounds was only 2.3% of the dose and 8.9% of the urinary radioactivity.

Keyphrases □ *l*-Bunolol—metabolism in rats, urinary metabolites identified □ Metabolism—*l*-bunolol in rats, urinary metabolites identified □ Antiadrenergic agents—*l*-bunolol, metabolism in rats, urinary metabolites identified

The β-adrenoceptor blocking activity of *l*-bunolol is now being evaluated by clinical trials. Earlier studies on bunolol metabolism were conducted in dogs (1–3) and *in vitro* (4, 5) with the *dl*-form. The present report is the first to describe the metabolism of the *l*-isomer, which is approximately 2.5 times more potent than the *dl*-preparation in inhibiting isoproterenol tachycardia in conscious dogs (6).

EXPERIMENTAL

Reference Compounds—Isotopically labeled *l*-bunolol hydrochloride contained tritium in the 7-position of the naphthalenone ring (69.3 μCi/mg, 99.9% radiochemical purity, 99.3% chemical purity). Synthetic nonradioactive dihydrobunolol, β-(5-oxytetralonyl)lactic acid, (5-oxytetralonyl)acetic acid, and ^{14}C-labeled hydroxybunolol and hydroxydihydrobunolol isolated from dog urine (3) were used.

Radioactivity Counting—Quantitative assays of urine for tritium were performed with a liquid scintillation spectrometer[1]. The external standardization method was employed for quench corrections. Feces were assayed for tritium after combustion in an oxidizer[2].

Animals, Dosing, and Collection of Excreta—Male Wistar rats[3], 270–290 g, were dosed by gavage with an aqueous solution of ^{3}H-*l*-bunolol hydrochloride (10 mg/kg). The animals were housed in individual glass

[1] Packard Tri-Carb model 3320.
[2] Oxymat JA-101, Teledyne Intertechnique, Westwood, N.J.
[3] Marland Breeding Farms, Hewitt, N.J.