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).

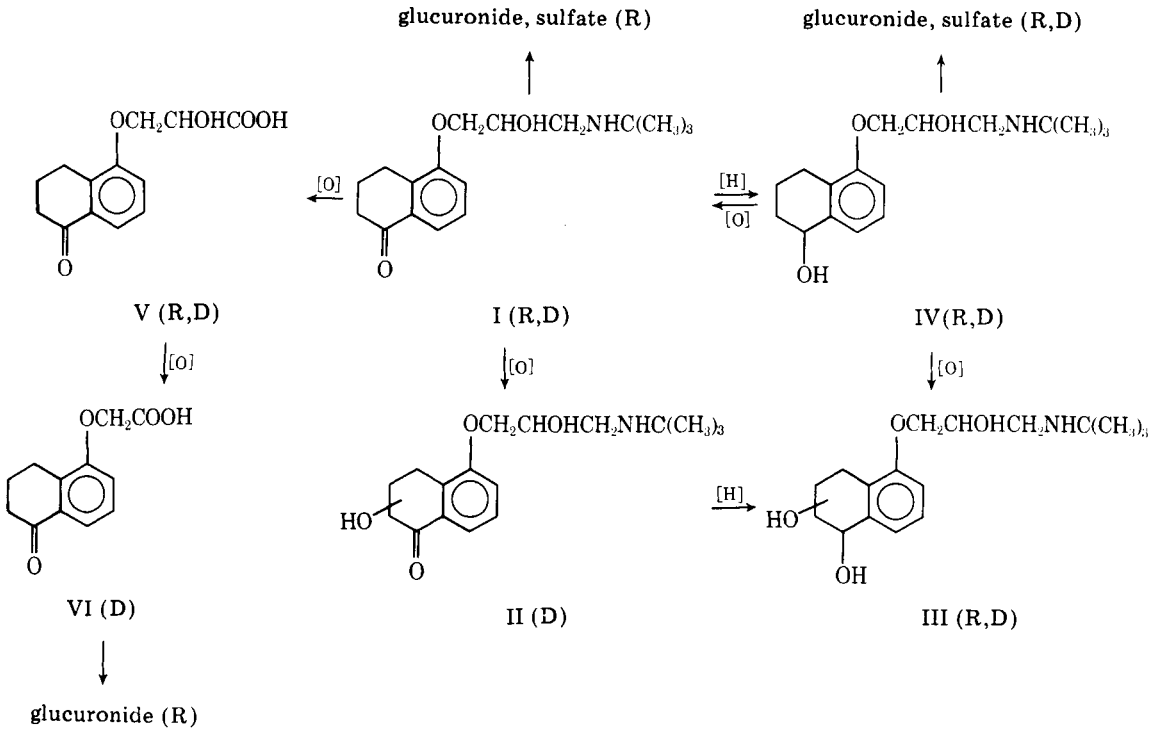
EXPERIMENTAL

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.



Scheme I—Pathways of bunolol biotransformation in the rat and dog; (R) and (D) signify the identification of the compound in rat and dog urine, respectively.

metabolic units and were offered a synthetic liquid diet[4] *ad libitum*. Urine and feces were collected from each rodent for 24 hr and assayed for tritium.

Chromatography—After developing the silica gel G[5] TLC plates in chloroform–acetic acid–methanol (15:1:4), radioactive bands were located with a radiochromatogram scanner[6]. For most purposes, areas under radioactive peaks were measured with a compensating planimeter[7].

Fractionation of Urine—The urine from the three rats was adjusted to pH 2 and extracted eight times with 500-ml volumes of ether to collect unconjugated acids. Unconjugated bases were extracted with ether (15 × 500 ml) after adjusting the pH of the aqueous phase to 10. Then the aqueous phase was brought to pH 5, freed of ether with a nitrogen stream, and incubated at 37° for 7 hr with β-glucuronidase[8] (0.2 IU/ml). Fresh enzyme (0.2 IU/ml) was added, and incubation was resumed for 17 hr.

After the solution was brought to pH 2, deconjugated acids were extracted with ether (5 × 500 ml). Basic aglycones were obtained by adjusting the aqueous phase to pH 10 and extracting eight times with ether. A nitrogen stream was used to remove ether from the aqueous phase before incubation at pH 6 with aryl sulfatase[9] (0.7 IU/ml), first for 6 hr and then for 18 hr, after adding the same quantity of fresh enzyme. Deconjugated acids were collected at pH 2 with ether, and deconjugated bases were extracted at pH 10. The remaining aqueous solution contained unclassified polar metabolites.

Fractionation of Bunolol and Metabolites—A 1.5 × 82-cm column of Sephadex G-10 was used for the fractionation of bunolol and its basic metabolites. After the column was equilibrated with 20 m*M* sodium phosphate buffer (pH 7.0), calibration was performed by applying 1-ml solutions of bunolol (0.1 μmole), dihydrobunolol (0.3 μmole), and hydroxydihydrobunolol (0.1 μmole) in phosphate. Effluents of radioactive samples were monitored for tritium, and those of nonradioactive compounds were evaluated by UV spectrometry (bunolol at 254 nm, dihydrobunolol at 272 nm).

After the separation of urinary components, the fractions eluted from ~75 to 108 ml were pooled and subsequently analyzed for dihydrobunolol. Similarly, according to the precalibration data with reference compounds (3), the fractions eluted from ~108 to 140 ml were pooled for the subsequent quantitative determination of bunolol. Each pool was reduced to dryness *in vacuo*. and the residues were taken up in methanol.

Aliquots from each pool were chromatographed on 20 × 20-cm silica gel G plates flanked by reference compounds. Each pool representing the eluate from ~75 to 108 ml was chromatographed in parallel with reference dihydrobunolol; each pool representing the eluate from ~108 to 140 ml was chromatographed in parallel with reference bunolol (10 μg/spot). After development of the plates, bunolol was visualized under UV light and dihydrobunolol was visualized by exposure to iodine vapor. The bands between the reference spots were scraped from the plates, and the tritium was eluted with methanol.

RESULTS

The mean excretion of the isotope in 24 hr was 39.0% of the orally administered ^{3}H-*l*-bunolol (13.2% in feces and 25.8% in urine). Solvent extraction and TLC indicated that the urine contained at least 31 labeled compounds (Table I). More than half of the urinary radioactivity consisted of unclassified polar metabolites. Base glucuronides comprised the largest classified fraction and represented 26.9% of the excreted radioactivity.

Table II shows the nine labeled compounds identified in rat urine and the quantities of each. The level of unchanged bunolol was very low, but bunolol glucuronide was present in the largest amount and some bunolol sulfate was detected. Dihydrobunolol showed a similar pattern; *i.e.*, it was present in the same three forms and predominantly as the glucuro-

Table I—Classification and Quantification of Labeled Components in Rat Urine after a Single Oral Dose of ^{3}H-*l*-Bunolol Hydrochloride (10 mg/kg)

Fraction	Labeled Components, Minimum Number	Percent of Dose	Percent of Urinary Tritium
Unconjugated acids	3	1.04	4.0
Unconjugated bases	12	2.58	10.0
Acid glucuronides	2	0.37	1.4
Base glucuronides	5	6.94	26.9
Acid sulfates	3	0.28	1.1
Base sulfates	5	0.92	3.6
Polar metabolites	1	13.70	53.1
Total	31	25.8	100

[4] Carnation Slender.
[5] Analtech.
[6] Packard model 7201.
[7] Keuffel and Esser.
[8] Ketodase, Warner-Chilcott.
[9] Calbiochem.

Table II—Quantities of Labeled Compounds Identified in the Urine of Rats after a Single Oral Dose of ^{3}H-*l*-Bunolol Hydrochloride (10 mg/kg)

Compound	Unconjugated		Glucuronide		Sulfate	
	% of Dose	% of Urinary Tritium	% of Dose	% of Urinary Tritium	% of Dose	% of Urinary Tritium
Bunolol	0.09	0.35	1.32	5.12	0.02	0.08
Dihydrobunolol	0.02	0.08	0.19	0.74	0.03	0.12
Hydroxybunolol	N.D.[a]	—	N.D.	—	N.D.	—
Hydroxydihydrobunolol	0.15	0.58	N.D.	—	N.D.	—
β-(5-Oxytetralonyl)lactic acid	0.19	0.74	N.D.	—	N.D.	—
(5-Oxytetralonyl)acetic acid	N.D.	—	0.29	1.12	N.D.	—

[a] Not detected.

nide. Hydroxydihydrobunolol was found, but hydroxybunolol was absent. The other metabolites were unconjugated β-(5-oxytetralonyl)lactic acid and (5-oxytetralonyl)acetic acid glucuronide. The total quantity of identified labeled compounds was only 2.3% of the dose and 8.9% of the urinary radioactivity.

DISCUSSION

It is very improbable that the great differences between bunolol biotransformation in the rat and dog (3) are attributable to the administration of the *l*-isomer to rats and the *dl*-form to dogs. Additionally, dogs excreted 62% of the dose in urine in the same period (1) as rats excreted only 26%; the slow excretion by the rat probably results from the extensive enterohepatic recirculation of bunolol metabolites[10]. More than half of the bunolol metabolites in rat urine are unclassified polar metabolites; such unknown compounds represent only 23% of the metabolites in dog urine (3). Whereas base glucuronides constitute the major classified fraction of bunolol metabolites in rat urine, they contribute much less to the radioactivity of dog urine where unconjugated acids and bases account for 61% of the isotope (3).

There are several qualitative differences in bunolol (I) biotransformation by rats and dogs. The most interesting is the presence of hydroxybunolol (II) in dog urine and its absence in rat urine. This finding implies that hydroxydihydrobunolol (III) is formed from dihydrobunolol (IV), not from hydroxybunolol, at least in the rat (Scheme I). Also, β-(5-oxytetralonyl)lactic acid (V) was present in both rat and dog urine, while (5-oxytetralonyl)acetic acid (VI) was detected only in dog urine.

[10] M. C. Crew, R. J. St. Hilaire, R. L. Gala, and F. J. Di Carlo, unpublished data.

The quantitative differences may have greater pharmacological significance. Dihydrobunolol has approximately the same β-adrenergic blocking potency as bunolol (4). The total quantities of unconjugated bunolol and dihydrobunolol were 0.7% of the dose in dog urine and only 0.1% in rat urine. These data suggested that bunolol might express its activity more readily in dogs than in rats, an interpretation verified experimentally (7).

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