Synthesis and Neuromuscular Blocking Activity of a 3,3′-Bisbufotenidine Analog

Keyphrases □ 3,3′-Bisbufotenidine analog—synthesized and screened as possible muscle relaxant, neuromuscular blocking activity □ Bufotenidines—synthesis and screening of 3,3′-bisbufotenidine analog, neuromuscular blocking activity □ Neuromuscular blocking activity—3,3′-bisbufotenidine analog, effect of size □ Muscle relaxants, potential—synthesis and screening of 3,3′-bisbufotenidine analog, neuromuscular blocking activity

To the Editor:

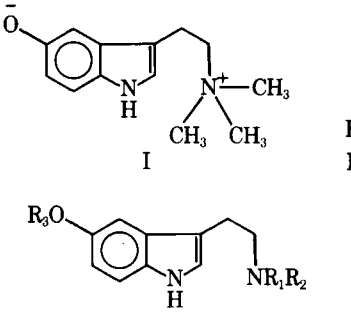
In correlating the neuromuscular blocking activity of some indole-3-alkylamines with their chemical structures, it was previously demonstrated (1–4) that the presence of two hetero atoms (O and N) in the molecule, intervened by six carbon atoms through the shortest route, is essential. It was also shown that the degree of activity, although not restricted to quaternary amines only, was a function of the pKa of the test compounds.

Accordingly, the neuromuscular blocking activity of bufotenidine (I) was found to be the most potent among the series of compounds (I–IV) tested. Bufotenidine itself, however, showed a significant difference in its activities *in vivo* and *in vitro*. Thus, the antiacetylcholine effect of I on the rectus abdominis muscle of the frog was almost equipotent to that of tubocurarine, while its effect on the reversal of neostigmine block on sciatic nerve–gastrocnemius muscle preparation of albino rats was five to six times less than the latter. The possibility that the size of the molecule may be responsible for this difference in activity was repeatedly considered theoretically (2–5) but not tested by observation. We have now obtained evidence to support this contention.

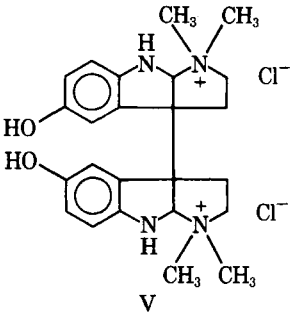
For these purposes, the bisbufotenidine analog (V) was synthesized from 5-*O*-benzylbufotenine (VI), and its neuromuscular blocking activity was tested both *in vitro* and *in vivo*. The synthesis was based on two previous reports: *(a)* that indolyl magnesium halides are ionic in tetrahydrofuran solution (6), and *(b)* that cyclization of an ethereal suspension of N_a-dipteryl magnesium iodide with anhydrous ferric chloride affords *rac*-chimonanthine (7).

Accordingly, 3-(2-*N*,*N*-dimethylaminoethyl)-5-benzyloxyindole (5-*O*-benzylbufotenine) (VI) was first subjected to ionization of N_a by formation of the Grignard reagent (VII), followed by oxidation with anhydrous ferric chloride to yield V as the major product. Methyl magnesium iodide was prepared from magnesium turnings (0.95 g) and methyl iodide (5.5 g) in anhydrous ether (300 ml) under a nitrogen cloud. A solution of 5-*O*-benzylbufotenine (11.5 g) in ether (500 ml) was added dropwise in 1 hr to the stirred solution of methyl magnesium iodide. The resulting suspension was stirred for a further 2-hr period, and then a solution of anhydrous ferric chloride (7 g) in ether (200 ml) was added dropwise.

The reaction was stirred for 12 hr and then treated with an aqueous solution of ammonium chloride to effect mild acidic hydrolysis of the product. The ethereal layer was separated, the aqueous solution was made basic (pH ~10), and the liberated bases were extracted with ethyl acetate. The gelatinous precipitate of ferrous hydroxide was adsorbed on powdered cellulose. The solids were removed by filtration, and the residue was again extracted with hot ethyl acetate. The total extractives (8.4 g), obtained from the ether and ethyl acetate extracts, revealed four major spots due to quaternary bases, in addition to the unreacted indolealkylamine (VI, 54%), on TLC analysis [silica gel G[1], benzene–ethyl acetate (4:1)].

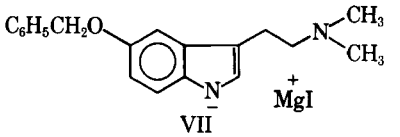


I



V

II: $R_1 = R_2 = CH_3$, $R_3 = H$
III: $R_1 = R_3 = H$, $R_2 = CH_3$
IV: $R_1 = R_2 = R_3 = H$
VI: $R_1 = R_2 = CH_3$, $R_3 = CH_2C_6H_5$



VII

The benzyl group was removed from the mixture of products by treatment with trifluoroacetic acid (20 ml) at room temperature for 18 hr (8). Column chromatography of the resulting mixture of phenolic bases over a magnesia silica gel[2] and a resin[3] gave the major product (1.72 g, 22%) as a microcrystalline solid. The compound, mp 210–214°, $C_{24}H_{32}N_4O_2Cl_2$ (elemental analyses and potentiometric titration), showed spectral properties, *e.g.*, UV: λ_{max} (ethanol) 238 (log ϵ 4.02) and 295 nm (log ϵ 3.61) [indicating the presence of a quaternary N_a—C—N_b^+ system since, on dequaternization with lithium aluminum hydride in tetrahydrofuran, the maxima were shifted to λ 248 and 307 nm (9)]; PMR (CD_3OD): δ 3.24–3.30 (12H, N^+—CH_3), 4.28–4.31 (2H, N—CH—N^+), and 6.8–7.4 (6H, Ar—H). The mass spectrum of the dequaternized product (phenolic tertiary base) showed, aside from the molecular ion peak at *m/e* 378 (14%), significant fragment ion peaks at *m/e* 188 (22), 187 (16), 146 (68), and 43 (base peak), characteristic of symmetrical scission of the dimeric indolic base. On the basis of these data, the major product was assigned Structure V.

The bisbufotenidine analog (V) exhibited the expected pronounced curariform activity on the rectus abdominis muscle of the frog, inhibiting acetylcholine-induced spasm without affecting the potassium

[1] E. Merck.
[2] Florisil.
[3] Amberlite CG-400 (Cl^-).

chloride-induced contractions. The inhibition was reversible and the spasmolytic ED_{50} was 0.82 μg/ml. Bufotenidine and tubocurarine, in parallel assays, showed spasmolytic ED_{50}'s of 1.45 and 1.38 μg/ml, respectively. Subsequently, the effect of the quaternary dimeric alkaloid (V) to hasten the reversal of tetanic response due to neostigmine (0.3 mg/kg iv) on sciatic nerve–gastrocnemius muscle preparation of albino rats (10) was studied. The response, in absence of the test compounds, started recovering in about 10–15 min and was almost completely recovered by 30–40 min. The reversal process was brought to completion by 10–15 min after the administration of bufotenidine (I, 0.2 mg/kg iv), bisbufotenidine analog (V, 0.01 mg/kg iv), or tubocurarine (0.015 mg/kg iv).

The curarimimetic activity of V was also tested in chicks according to the method described by Lewis (11). The test compounds were injected intravenously through the alar vein in groups of 10 chicks. All three compounds showed flaccid paralysis of the limbs with flexion of the head of chicks in doses of 50, 4, and 10 μg for I, V, and tubocurarine, respectively. From these results, it appears that the bisbufotenidine analog could be of potential therapeutic importance as a muscle relaxant. The nominal side effects encountered during its routine pharmacological screening may not pose any serious obstacle to its possible use as an adjuvant to anesthesia.

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BOOKS

REVIEWS

Pharmaceutical Calculations. By JOEL L. ZATZ. Wiley, 605 Third Ave., New York, NY 10016, 1973. 311 pp. 17 × 25.5 cm. Price $7.95.

Using the format of linear programming with occasional branched remedial sequences, this book adequately presents the most important aspects of pharmaceutical calculations. The book actively involves the student in the learning process by requiring frequent responses and solutions to short questions and problems. The material is presented in 11 chapters covering the interconversion of pharmaceutical systems of weights and measures, elementary prescription notation and drug dosing, formula adjustment, calculation of percentages and other expressions of concentration, stock solutions, dilutions and concentrations, and milliequivalents. Zatz is careful and thorough in his presentation of unit labels in the basic review of mathematical operations and in his treatment of scientific notation. Accuracy of measurement using pharmaceutical equipment is conveniently linked to a presentation of factors affecting percent error and ways of determining significant figures.

The first seven chapters contain useful concise statements of objectives and goals, usually with some indication of the competence the student may be expected to achieve from a study of each chapter. The last four chapters are not consistent in their introductions, thereby losing some of the cohesiveness of the book. Furthermore the use of a cumbersome and oftentimes confusing symbol for minim, coupled with the dual use of the letter j as a general symbol for an unknown quantity as well as the final "i" in a roman numeral, represents typesetting shortcomings of this first edition that can seriously mislead all but the most astute students. For all of his intent to present problems and methods in a logical straightforward manner, the author makes the student unnecessarily dependent upon the arbitrary use of ratios and proportions in a large number of problem groups.

While the book may be helpful to some students undertaking individualized or self-paced study in pharmaceutical calculations, it will be most useful in a course where it finds companion explanation and clarification by the professor, particularly in the areas of methods and rationale for the calculation of apothecary percent w/v and ratio strength. The book can function as a helpful guide and workbook for the student, but its contents may require supplementary problem sets for mastery. The book is generally effective in its scope, variety of problems, and presentation style. It