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Notes

Bufotenine Esters

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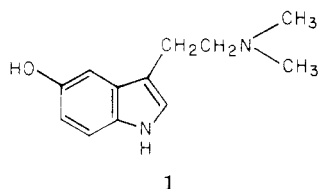
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Bufotenine (5-hydroxy-*N,N*-dimethyltryptamine) has been reported to be behaviorally inactive or only very weakly active in man and animals; this may be a consequence of its low partition coefficient and resultant inability to penetrate the blood-brain barrier. The acetyl, propionyl, butyryl, isobutyryl, and pivalyl esters of bufotenine were prepared for future pharmacological evaluation. Unexpectedly, it was found that these esters all possess a relatively high affinity for the serotonin receptors of the isolated rat stomach fundus preparation. A semiquantitative chromatographic measurement of ester hydrolysis suggests that extensive hydrolysis of the esters to bufotenine does not occur under the conditions of the affinity assay

Bufotenine (5-hydroxy-*N,N*-dimethyltryptamine, 1) has



been reported to be hallucinogenic in man.^{2a} This finding has been refuted,^{2b} however, and it is now commonly held that the psychotomimetic activity of bufotenine is questionable. It has been suggested that, because of poor lipid solubility, appreciable amounts of administered bufotenine do not penetrate the blood-brain barrier.^{3,4} In addition, attempted administration of larger doses of 1 to human subjects produces peripheral effects which may mask or obscure any central effects.^{2b} Indeed, bufotenine is not very lipid soluble, as reflected by its partition coefficient.⁵ The inability of 1 to penetrate the blood-brain barrier has also been demonstrated experimentally by measurement of animal brain levels, at various time intervals, following the administration of bufotenine.^{3,4}

The question which now arises is whether 1 is intrinsically inactive or whether it is an active compound which simply does not get into the brain. This question is of more than mere academic significance, since enzyme systems which can convert the neurotransmitter serotonin to 1 have now been identified.⁶ In addition, though still a subject of controversy,^{7,8} bufotenine and other methylated tryptamines have been detected in the urine of schizophrenics.⁹⁻¹² To this extent, Sanders and Bush³ have found that, in rats, only 6% of an administered dose of 1 is excreted unchanged; even detection of small amounts in the urine of schizophrenic patients might thus be quite significant.

There is indirect evidence that 1 is behaviorally active. For example, Mandel¹³ and Geyer et al.¹⁴ have found 1 to be at least equiactive with the hallucinogen 5-methoxy-*N,N*-dimethyltryptamine (5-OMe-DMT) when administered to animals via intraventricular injection, thereby bypassing the blood-brain barrier. Newborn (24-48-h old) chicks possess a poorly developed blood-brain barrier, and Rauzzino and Siefert¹⁵ have noticed that administration of bufotenine to such animals results in responses typical of those of other established hallucinogens, such as *N,N*-dimethyltryptamine (DMT). Gessner and Dankova⁴ administered the more lipid-soluble 5-acetoxybufotenine to mice, where it is hydrolyzed, presumably by brain tissue esterases, to bufotenine. Measuring LSD-like activity as a function of brain concentration, the order of potency was found to be bufotenine > 5-OMe-DMT > DMT. Glennon et al.¹⁶ have recently reported that several potent tryptamine and phenalkylamine hallucinogens possess high affinities for the serotonin receptors of the isolated rat fundus preparation, and Glennon and Gessner¹⁷ had previously reported bufotenine to possess twice the affinity of 5-OMe-DMT.

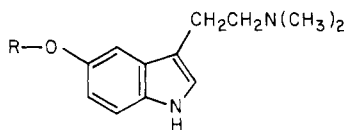
It was thus of interest to prepare several esters of bufotenine for eventual pharmacological evaluation. We now report a series of five such esters, along with their serotonin receptor binding affinities.

Results and Discussion

The esters 2-6 were prepared by direct acylation of bufotenine and are listed in Table I. Bufotenine itself possesses a high affinity for serotonin receptors. In order to ascertain whether the ester function serves simply as a protecting group or whether the bufotenine esters themselves might possess some affinity, serotonin receptor-binding affinities were determined and are also

Table I. Bufotenine Esters and 5-HT Receptor Affinity Data^a

no.	R	mp, ^b °C	formula	anal.	pA ₂ ^c	slopes ^d	partit coeff ^e
2	-COCH ₃	158-161 ^f	C ₁₆ H ₂₀ N ₂ O ₆		7.71 (±0.07), 3	1.15, 1.05, 1.06	2.31
3	-COCH ₂ CH ₃	163-164	C ₁₇ H ₂₂ N ₂ O ₆	C, H, N	7.27 (±0.02), 2	0.84, 0.96	5.60
4	-COCH ₂ CH ₂ CH ₃	166-167	C ₁₈ H ₂₄ N ₂ O ₆	C, H, N	7.32 (±0.10), 2	1.02, 1.20	19.70
5	-COCH(CH ₃) ₂	186-188	C ₁₈ H ₂₄ N ₂ O ₆	C, H, N	7.40 (±0.09), 2	1.10, 1.11	19.00
6	-COC(CH ₃) ₃	185-186	C ₁₉ H ₂₆ N ₂ O ₆	C, H, N	7.42 (±0.02), 2	0.94, 1.16	
7	<i>N,N</i> -dimethyltryptamine (DMT)				6.00 ^g		2.61 ^g
8	5-methoxy- <i>N,N</i> -dimethyltryptamine (5-OMe-DMT)				7.08 ^g		3.30 ^g
1	5-hydroxy- <i>N,N</i> -dimethyltryptamine (5-OH-DMT)				7.41 ^g		0.06 ^g



^a Serotonin, pD₂ = 7.45 (±0.35), 33; i.e., K_A = ~35 nM. ^b All compounds are reported as their hydrogen oxalate salts and were recrystallized from absolute ethanol (compound 5 recrystallized from 95% EtOH). ^c pA₂ values are followed by standard deviation and number of determinations. ^d Negative slopes of Schild plots. ^e Chloroform/aqueous buffer (pH 7.4) partition coefficients were determined as previously reported (ref 5 and 22). Insufficient sample for compound 6. ^f Elemental analysis previously reported. ^g Data from Glennon and Gessner (ref 17 and 22).

listed in Table I. From the data it appears that all of the bufotenine esters possess a receptor affinity similar to that of bufotenine itself. This finding is rather surprising in view of the fact that simple methylation of the phenolic hydroxyl group of 1 (i.e., 8) results in a twofold decrease in affinity.

Although hydrolysis could not account for the high affinity of 2, it is entirely possible that some of the esters could be hydrolyzed to bufotenine during the course of the affinity assay. This possibility was investigated by a semiquantitative measurement of ester hydrolysis using a chromatographic method developed by Narasimhachari and Plaut.¹⁸ A maximum of 5% (2-4), though usually approximately 1% (5 and 6), ester hydrolysis occurs in Tyrode's solution upon incubation at 37 °C for 10-15 min. When the incubation is carried out in the presence of freshly prepared fundus strip, simulating the affinity assay conditions, a greater degree of hydrolysis occurs. With the exception of 6, for which less than 5% hydrolysis occurs, all of the esters are hydrolyzed to the extent of 15-25%. Because the hydrolysis studies employed incubation times longer than that actually used in the affinity assay, it is reasonable to assume that no greater than 25% ester hydrolysis is occurring.

It is evident that all of the esters possess an appreciable affinity for the serotonin receptors of the rat fundus preparation. The ester function can alter the electronic nature of the indolic ring or, perhaps, interact directly with some region associated with the serotonin receptors. Nevertheless, a certain degree of bulk tolerance is exhibited, particularly with the sterically hindered ester 6. Compound 2 has been shown to undergo hydrolysis *in vivo* but not before a considerable quantity has reached the brain.⁴ In light of the finding that potent hallucinogens possess a high affinity for serotonin receptors,^{16,22} it is intriguing to speculate about the potential activity of compound 6. Indeed, preliminary animal studies, employing rats, indicate 6 to be active. Using a discriminative stimulus assay, the ester 6 is one-fifth as active as, but can produce behavioral effects (i.e., interoceptive cues) similar to, those produced by 5-OMe-DMT. In other words, a dose of approximately 25 μmol/kg (*n* = 6) of 6 will generalize to a 5-OMe-DMT training dose of 5 μmol/kg (unpublished data).

All of the esters examined are more lipid soluble, as determined by their partition coefficients (Table I), than bufotenine itself. The simpler esters, depending on their

rates of hydrolysis which have yet to be determined, might be used to deliver bufotenine to the brain in order to study its behavioral effects. The more sterically hindered esters, such as 6, can be used to evaluate the behavioral effects of acylated bufotenine analogues and to explore regions of potential bulk tolerance which might be associated with serotonin receptors. Such studies are now underway in these laboratories.

Experimental Section

Nuclear magnetic resonance (¹H NMR) spectra were recorded using a Perkin-Elmer R-24 spectrometer and mass spectra were obtained employing a Finnigan 4000 GC-MS data system. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected.

General Method of Ester Synthesis. Bufotenine (20 mmol) was dissolved in aqueous NaOH (10%, 11.5 mL) and the solution cooled to 5 °C. Acid anhydride (20 mmol) was added dropwise with stirring, while the temperature was maintained below 10 °C. When the addition was complete, NaOH (10%, 10 mL) was added and the reaction mixture was stirred at 5-10 °C for an additional 10 min. The precipitate was collected and extracted with CHCl₃ (6 × 15 mL), and the combined CHCl₃ extracts were washed with water (2 × 15 mL), dried (Na₂SO₄), and evaporated to dryness. An ether solution of oxalic acid was added dropwise to a stirred ether solution of the crude bufotenine ester to yield the hydrogen oxalate salt in nearly quantitative yield. Melting points and analytical data are shown in Table I. Unlike bufotenine, all of the esters gave a negative nitrosonaphthol reaction,¹⁹ indicative of the absence of an unsubstituted indolic hydroxyl group.

Binding-Affinity Studies. Sprague-Dawley rats, of either sex, weighing 200-500 g were used. The rat stomach fundus preparation employed was essentially that of Vane,²⁰ with the previously described modifications.¹⁶ Two strips were cut from the same tissue and used in parallel 8-mL muscle baths. The relative sensitivity of the two strips was determined, after a 1-h equilibration period, by the use of 5-hydroxytryptamine (5-HT) doses giving submaximal contractions. Only one compound was tested per preparation, the second strip serving as control.

The ability or potency of each agent to inhibit the contractile response to 5-HT was determined by obtaining cumulative dose-response curves to 5-HT, first in the absence of the agent in question and, then, in the presence of increasing concentrations thereof. Each compound was tested at four or five concentrations on each strip. Concentrations of compound usually ranged from 0.1 to 3 μM, and eight to ten doses of 5-hydroxytryptamine hydrogen oxalate, ranging from 1 nM to about 10 μM, were used to generate the dose-response curves. (The compounds themselves did not produce an agonistic response at the concentrations tested; however, an agonistic response could be obtained at higher

concentrations.) The dose-response curves obtained were subjected to probit analysis, using the Fortran program ISOBOL, to compare parallelism of slopes; the slopes of the common regression line of the response metameter on the logarithm of the dose and the 5-HT concentrations required for half-maximal contraction were calculated. The apparent affinities were calculated as pA_2 values by the method of Arunlakshana and Schild.²¹

Hydrolysis Experiments. In order to determine the extent of ester hydrolysis, solutions of the esters in Tyrode's buffer were incubated at 37 °C for 10–15 min, both with and without a freshly cut portion of fundus strip (ca. 1 g wet weight). At the end of the incubation time, the fundus strip was immediately removed. The amount of bufotenine present was determined by a modification of the thin-layer chromatographic procedure of Narasimhachari and Plaut.¹⁸ The procedure involved spotting 1–5 μ L of the ester solutions on silica gel G TLC plates along with standard quantities of bufotenine. After development with $CHCl_3$ -MeOH-NH₄OH (12:7:1) and spraying with *o*-phthalaldehyde reagent, the size and fluorescence intensity of the bufotenine spots from the ester solutions were compared with the standard spots in order to obtain quantitative estimates of the amount of hydrolysis.

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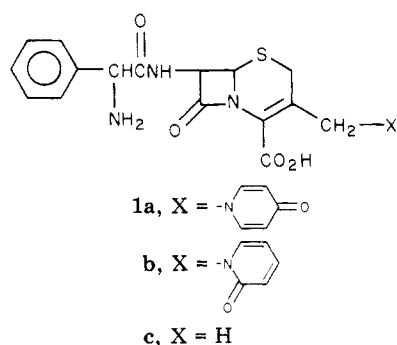
Cephalosporin Derivatives with 2- and 4-Pyridone Groups at Carbon-3

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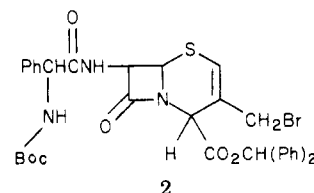
Two compounds, analogues of cephalixin with 2- and 4-pyridone groups at C-3, were prepared. Biological evaluation found the compounds to exhibit activity against Gram-positive and Gram-negative organisms in vitro and in vivo. The compounds were only active in vivo on subcutaneous administration.

One approach to the design of orally effective cephalosporins has been the modification of C-3 methylene of cephalixin (1c). The phenylglycine group at C-7 seems

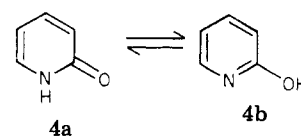


a requisite for oral activity in cephalosporin antibiotics. This paper reports the synthesis and activity of compounds incorporating the 2- and 4-pyridone moiety at C-3 of cephalixin (1a,b).

As a general route to these compounds we decided on the reaction of the allylic bromide 2 with the trimethylsilyl



ether of a hydroxy-substituted pyridine.^{1a,b} The nitrogen atom of 2-hydroxypyridine is amide-like due to the predominance of the keto form 4a in the equilibrium 4a-4b.²



The nonbasic character of 2-hydroxypyridine accounts for