mixed melting-point determination with a known sample and their identical IR spectra.

Anal.—Calc. for $C_{31}H_{64}$: C, 85.31; H, 14.68. Found: C, 85.52; H, 14.46.

Hentriacontanol—Elution with a benzene–hexane mixture (1:1) yielded a saturated and optically inactive compound (540 mg.), m.p. 82–84°, identified as hentriacontanol. The IR spectrum showed peaks at 3550 (—OH) and 800 cm.$^{-1}$ (—CH_2).

Anal.—Calc. for $C_{31}H_{64}O$; C, 82.30; H, 14.16. Found: C, 82.42; H, 14.32.

Hentriacontanol Acetate—This was prepared as usual and crystallized from acetone as colorless flakes, m.p. 74–76°. It was analyzed for $C_{33}H_{66}O_2$.

β-Sitosterol—The benzene eluate furnished free β-sitosterol (350 mg.), m.p. 135–137°, identified *vide supra*.

The alcoholic extractive was finally extracted with hot distilled water (2 l.). The aqueous extract was treated with lead acetate solution (5%) and filtered. The precipitated lead complex was suspended in alcohol and decomposed with hydrogen sulfide gas. The sulfide was filtered, and alcohol was removed under reduced pressure. Because it did not yield any crystalline product, the concentrate was hydrolyzed with alcoholic hydrochloric acid (7%) and worked up as usual. The hydrolysate showed two spots on the TLC plate, corresponding to kaempferol and quercetin.

Kaempferol—The hydrolysate was dissolved in aqueous borax solution (0.5%) and exhaustively extracted with ethyl acetate. The ethyl acetate fraction, on crystallization from dilute ethanol, gave a yellow crystalline substance (350 mg.), m.p. 274–276°. This was identified by mixed melting point and cochromatography with an authentic sample of kaempferol.

Anal.—Calc. for $C_{15}H_{10}O_6$; C, 62.9; H, 3.49. Found: C, 62.68; H, 3.38.

It formed a tetraacetate as silky needles, m.p. 181–183° (fused sodium acetate and acetic anhydride; 4 hr., 130°) and tetramethyl ether, m.p. 150–152° (dimethyl sulfate and anhydrous potassium carbonate in the presence of acetone).

Quercetin—The remaining borax-soluble fraction was decomposed with dilute hydrochloric acid and extracted with solvent ether. On removal of the solvent and crystallization from dilute methanol, a microcrystalline yellow substance (700 mg.), m.p. 308–310°, was obtained; UV absorption: $\lambda_{max.}^{ethanol}$ 275 (log ε 4.33) and 375 nm. (log ε 4.32).

Anal.—Calc. for $C_{15}H_{10}O_7$: C, 59.60; H, 3.31. Found: C, 59.5; H, 3.42.

It showed no depression in melting point with a known sample of quercetin. The compound formed a pentaacetate, m.p. 193–195°, and a pentamethyl ether, m.p. and mixed m.p. 152–153°.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 19, 1971, from the *Department of Chemistry, Catholic University of America, Washington, D.C., and the †Indian Veterinary Research Institute, Izatnagar, India.

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Naturally Occurring and Synthetic β-Carbolines as Cholinesterase Inhibitors

S. GHOSAL*▲, S. K. BHATTACHARYA†, and R. MEHTA*

Abstract □ Inhibitory activities of a number of naturally occurring and synthetic aromatic and tetrahydro-β-carbolines toward true and pseudocholinesterases were investigated. The potencies of the active compounds were measured and compared with those of physostigmine by their ability to protect acetylcholine from the cholinesterase (assayed on frog *rectus abdominis muscle*) and by using the chromodacryorrhea test in albino rats. Some quaternary aromatic β-carbolines were found to be about one-sixth as potent as physostigmine. The inhibitory potencies were nearly the same against acetylcholinesterase and pseudocholinesterase. The tertiary aromatic β-carbolines showed only weak activity, while the tetrahydro-β-carbolines lacked this activity. Influence of indole-*N*-methylation on the inhibitory activity was greater with the quaternary β-carbolines than with the tertiary series. The activity also depended on the degree of extended conjugation of the inhibitor molecule. Other factors involved in the anticholinesterase activities of the β-carbolines were examined, and a plausible mechanism of action was proposed.

Keyphrases □ β-Carbolines, natural and synthetic—as cholinesterase inhibitors, pharmacological evaluation, mechanism of action □ Cholinesterase inhibitors—pharmacological evaluation of naturally occurring and synthetic β-carbolines, mechanism of action □ Anticholinesterase activity—pharmacological evaluation of naturally occurring and synthetic β-carbolines, mechanism of action

Although considerable work has been done on naturally occurring anticholinesterases (1), comparatively fewer investigations have been made so far on the possible anticholinesterase activities of β-carbolines. In two recent papers, we sought to rationalize the reported (2) uses of the bark and fruit extracts of two *Desmodium* species—*viz.*, *D. pulchellum* and *D. gangeticum* (Family Leguminosae), in the Indian system of medicine for eye diseases and certain intestinal malfunctions with the potent anticholinesterase activities of their total alkaloids (3, 4). The present paper reports the anticholinesterase activities of some individual β-carbolines isolated from the above plant parts and those of their synthetic analogs. In addition, anticholinesterase activities of a number of complex indole alkaloids, containing an aromatic β-carboline

Table I—Inhibitions of Cholinesterases by β-Carbolines[a]

Compound	Molecular weight	Amount of Inhibitor Required for 50% Acetylcholine Protection, moles × 10^{-5}	Amount of Inhibitor Required for Positive Chromodacryorrhea Test, mg./kg.
I	182	4.1	1.75
II	196	6.5	2.25
III	212	2.7	1.08
IV	196	30.8	15.52
V	210	—	—
VI	170	43.9	16.41
VII	184	11.2	3.50
VIII	184	8.8	2.95
IX	198	7.1	2.50
X	188	—	—
XI	202	—	—
XII	203	—	—
XIII	217	—	—
XIV	188	+	—
XV	348	1.08	0.68
C_{20}-epimer of XV		1.17	0.74
XVI	358	—	—
XVII	272	0.89	0.61
XVIII	269	0.3	0.1

[a] — denotes that activity is absent; + denotes that activity appears at a very high dose.

moiety, were examined. The results are used to propose a probable mechanism of the β-carboline–enzyme interaction.

EXPERIMENTAL

Five of the 18 test compounds (I, II, III, VI, and X) were isolated from the fruits and barks of *D. pulchellum*[1] and *D. gangeticum*[1] following previously described procedures (3, 4). The tetrahydro-β-carbolines, XI–XIII, were prepared by the condensation of dipterine and formalin (or paraldehyde) in acidic media (5). The remaining indole-NH aromatic β-carbolines were prepared from tryptophan or abrine (6) according to the method of Perkin and Robinson (7). Indole-*N*-methylharman was obtained by heating ajmaline with soda lime at 360° (8). For Compound IV, indole-*N*-methylation was carried out according to the procedure of Potts and Saxton (9). The quaternary iodides were converted to the chlorides by passing their ethanolic solutions over De-Acidite E (Cl^-) (10). Alstonine, alstoniline, sempervirine, and serpentine were also used[2].

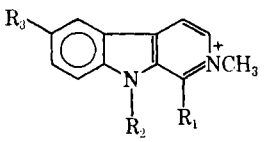
The anticholinesterase activities were investigated and compared with those of physostigmine, using *in vitro* and *in vivo* methods. *In vitro* studies were conducted by the biological assay technique of Hemsworth and West (11). Blood was collected from a dog with heparinized syringes. After centrifugation, the red blood cells were washed with 0.9% saline and hemolyzed in a volume of distilled water corresponding to the initial volume of plasma. A known concentration of acetylcholine (10 mcg. in 2 ml.) was added to the plasma (1 ml.) or hemolyzed red blood cells (1 ml.) and incubated at 37° for 30 min. in the presence and absence of different doses of physostigmine and the β-carbolines. Acetylcholine was completely hydrolyzed by the pseudocholinesterase present in the plasma and acetylcholinesterase in the red blood cells within this period in the absence of an inhibitor. In the presence of an inhibitor, the residual acetylcholine was biologically assayed on a frog rectus abdominis muscle preparation by the method of Richter and Crossland (12). The amounts of physostigmine and the β-carbolines required to produce 50% protection to acetylcholine from the cholinesterases were determined as the means of three experiments. The results are recorded in Table I.

In vivo studies were conducted by the chromodacryorrhea test (13) n albino rats. Acetylcholine (2 mg./kg. s.c.) was injected into albino rats (100–150 g.), and those producing red tears within 10 min. were used. Physostigmine and the β-carbolines were injected intraperitoneally in different doses to groups of rats 30 min. before injection of a smaller dose of acetylcholine (0.2 mg./kg. s.c.). The animals were observed for the appearance of red tears at 2-min. intervals for 14 min. The doses of physostigmine and the β-carbolines required to produce a positive response in all of the animals were determined as the means of three experiments. The results are recorded in Table I.

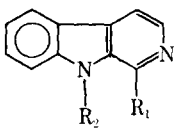
RESULTS AND DISCUSSION

Anticholinesterase activities of 18 naturally occurring and synthetic β-carbolines (I–XVII and the C_{20}-epimer of XV) were investigated, and the potencies of the active compounds were compared with those of physostigmine (XVIII). The results are recorded in Table I.

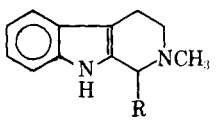
The results reflect that the aromatic quaternary β-carbolines (I, III, the two epimeric XV's, and XVII) are moderately strong inhibitors of both acetylcholinesterase and pseudocholinesterase. The inhibitory potencies are nearly the same against both enzymes. A considerable difference exists in the inhibitory potencies of the quaternary β-carbolines and their tertiary analogs (VI–IX), with the latter compounds exhibiting only weak activity. The tetrahydro-



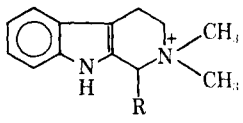
I: $R_1 = R_2 = R_3 = H$
II: $R_1 = CH_3, R_2 = R_3 = H$
III: $R_1 = R_2 = H, R_3 = OCH_3$
IV: $R_1 = R_3 = H, R_2 = CH_3$
V: $R_1 = R_2 = CH_3, R_3 = H$



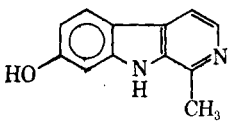
VI: $R_1 = R_2 = H$
VII: $R_1 = CH_3, R_2 = H$
VIII: $R_1 = H, R_2 = CH_3$
IX: $R_1 = R_2 = CH_3$



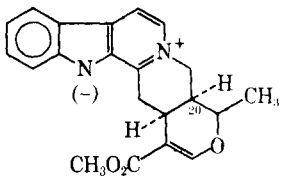
X: R = H
XI: $R = CH_3$



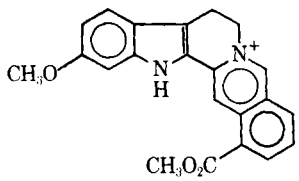
XII: R = H
XIII: $R = CH_3$



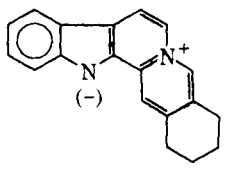
XIV



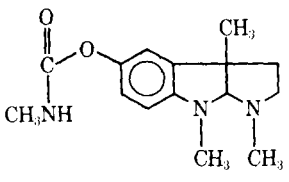
XV: alstonine



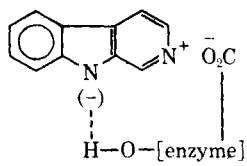
XVI: alstoniline



XVII: sempervirine



XVIII



XIX: inhibitor–enzyme complex

[1] The plants were identified and supplied by Mr. R. Sarkar. The voucher specimens are available from United Chemical and Allied Products, Calcutta-1, India.

[2] Obtained through Professor (Mrs.) A. Chatterjee, Calcutta University, India.

β-carbolines, both quaternary and tertiary (X-XIII), completely lack the inhibitory activity. The influence of indole-*N*-methylation on the anticholinesterase activity is greater with the quaternary aromatic β-carbolines than with the tertiary series. The activity is either absent or greatly reduced in indole-*N*-methylated quaternary β-carbolines. An oxygen function at C-6 (as in III) potentiates the inhibitory activity, but oxygenation at C-7 (XIV) considerably reduces it. The effect of extended conjugation on the anticholinesterase activities is revealed by the greater potency of sempervirine (XVII) compared to either alstonine (XV) or serpentine (C_{20}-epimer of alstonine). In the case of nonaromatic β-carbolines, *e.g.*, alstoniline (XVI), the degree of conjugation is without any effect.

These observations seem to indicate that the primary structural requirement for the anticholinesterase activities of the β-carbolines is a resonance stabilized-type (XIX) species. The lack of inhibitory activity noted with the tetrahydro-β-carbolines suggests a limited affinity of the molecule for the enzyme, the binding being only at one site, *i.e.*, tertiary or quaternary nitrogen. In the case of the quaternary aromatic β-carbolines a two-point inhibitor–enzyme attachment (XIX) could be visualized: one at the anionic site between the enzyme carboxyl group and the quaternary nitrogen of the inhibitor and the other at the esteratic site in the form of hydrogen bonding by the hydroxyl of the enzyme serine and the anionic indolic nitrogen. Other forces may also be operative, including the degree of extended conjugation of the inhibitor molecule. Thomas and Marlow (14) reported that there was a regular increase in the inhibitory potencies of the "aromatic-type" compounds (*i.e.*, compounds containing quaternary nitrogen in an aromatic ring) with the increase in the conjugated chain length. The phenomenon can be explained in terms of charge delocalization and favorable stereochemistry for binding of the inhibitor to the enzyme. A parallel was observed in the greater anticholinesterase potency of sempervirine (XVII) compared to either alstonine (XV) or serpentine.

The almost equal potencies noted for alstonine and serpentine suggest that the stereochemistry of the D/E rings of the indole alkaloids is without any influence on the anticholinesterase activities of the β-carbolines.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 10, 1971, from the *Department of Pharmaceutics, Institute of Technology, and the †Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-5, India.

Accepted for publication February 16, 1972.

R. Mehta is indebted to the C.S.I.R., New Delhi, India, for a research fellowship during the tenure of this work.

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COMMUNICATIONS

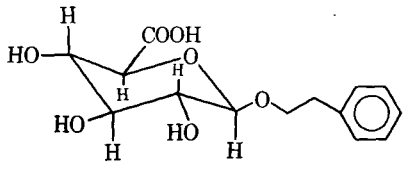
Direct Synthesis of a Glucuronide: Specific Complexing of Copper among Biological Cations

Keyphrases □ Glucuronides—direct synthesis of phenethyl-β-D-glucopyranosiduronic acid □ Phenethyl-β-D-glucopyranosiduronic acid—direct synthesis, complexation with copper □ D-Glucuronic acid—synthesis of phenethyl alcohol glucuronide □ Copper complexation—with phenethyl-β-D-glucopyranosiduronic acid

Sir:

The synthesis of glucuronides ordinarily requires several steps with frequently poor yields. I wish to report a synthesis of phenethyl-β-D-glucopyranosiduronic acid (I) in 52% yield directly from the alcohol and D-glucuronic acid.

A mixture of 30.0 ml. of phenethyl alcohol (0.25 mole) and 10.0 ml. of methanesulfonic acid was cooled to room temperature. To this was added 9.7 g. of D-glucuronic acid (0.05 mole); the slurry was stirred at room temperature for 7.5 hr., during which time most of the D-glucuronic acid dissolved. (Under comparable conditions, methanol reacted in 0.5 hr. and phenol in 2 hr., although in these instances the products were not isolated.) The nearly clear mixture was added to 200 ml. of 1 *M* sodium bicarbonate and extracted with ethyl acetate. Evaporation of the ethyl acetate at 25° in vacuum gave an oily residue. At this point the preparation was relatively clean, since TLC using ethyl acetate and silica gel showed only two components: a major one at R_f 0.57 and a minor one at R_f 0.73. The presumed esteruronide was hydrolyzed by stirring with a solution of 7.90 g. (0.025 mole) of barium hydroxide octahydrate in 200 ml. of water for 0.5 hr. Barium was removed by



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