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STEREOCHEMICAL CONSIDERATIONS IN RELATION TO THE PHARMACOLOGICAL ACTIVITY OF *PTEROTABERNA* ALKALOIDS

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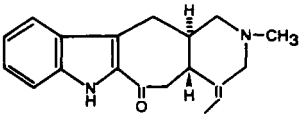
ABSTRACT.—Five 2-acylindole alkaloids, isolated from the leaves of *Pterotaberna inconspicua*, were pharmacologically investigated. The major alkaloids methuenine and 16-epimethuenine showed some interesting pharmacological features, whereas the minor alkaloids including 6-oxomethuenine and methuenine *N*-oxide were almost devoid of these pharmacological properties. Methuenine, being the most potent, was characterized as a non competitive antagonist against acetylcholine ($pD'_2=5.10\pm0.11$) and histamine ($pD'_2=5.13\pm0.14$) in guinea-pig ileum. Its potency was comparable to that of papaverine. 16-Epimethuenine behaved as a weak antihistaminic ($pA_2=6.55\pm0.08$). The stereochemistry of both components is discussed in relation to their pharmacological activity.

Pterotaberna inconspicua Stapf. (Apocynaceae) (1) grows as a shrub in Central Africa. The leaves are used in Zairese traditional medicine to treat hypertension, gastrointestinal upsets, and several kinds of aches.[1] Although aqueous extracts of the leaves are frequently employed, their chemical constituents have never been determined. Recently, the alkaloidal contents of the seeds and the root-bark of this plant have been explored (2), and the isolation and identification of five 2-acylindole alkaloids from the leaves of *P. inconspicua* have been carried out (3). This paper deals with the first pharmacological characterization of the major alkaloids, including methuenine and 16-epimethuenine, present in the leaves of this plant. More particularly their anticholinergic antihistaminic, and spasmolytic properties were investigated.

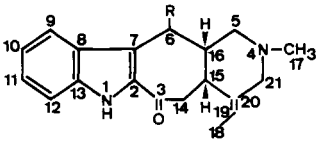
EXPERIMENTAL

PLANT MATERIAL.—The leaves of *P. inconspicua* were collected in Western Zaire and identified by Dr. P. Bamps, National Botanical Garden, Meise, Belgium. Voucher specimens are kept at the Department of Pharmaceutical Sciences, University of Antwerp, Antwerp, Belgium.

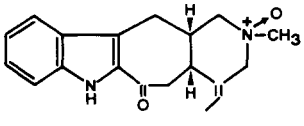
ISOLATION OF ALKALOIDS.—The following alkaloids were isolated from the leaves of *P. inconspicua* according to the procedure described elsewhere (3), and the yield was calculated on the dried leaves: methuenine (0.304%), 16-epimethuenine (0.160%), methuenine *N*-oxide (0.022%), and 6-oxomethuenine (0.020%). Structural formulas are shown in Figure 1.



16-epimethuenine



methuenine R=H,H
6-oxomethuenine R=O



methuenine-*N*-oxide

FIGURE 1. Structures of the four alkaloids isolated from the leaves of *Pterotaberna inconspicua*.

[1]Bakana P., Professor of Organic Chemistry, School of Pharmacy, Université de Kirhasa, Zaïre, personal communication (1 September 1979).

CHEMICALS AND SOLUTIONS.—Solutions of acetylcholine (Merck), histamine diHCl (Pharmachemic), and naloxone (Endo laboratories) were prepared daily. The concentrated solutions were diluted with 0.9% NaCl. PGE_2 (Upjohn) was stored at $-30°$ as a stock solution in absolute EtOH (1 mg/ml) and was diluted at the moment of assay with 0.9% NaCl.

The alkaloids were dissolved in 0.25 N HCl, yielding a 1 mg/ml solution, except for methuenine *N*-oxide, which was dissolved in absolute EtOH in order to obtain a concentration of 10 mg/ml. Stock solutions were stored at $-30°$ and portions were diluted at the moment of assay.

CUMULATIVE DOSE-RESPONSE EXPERIMENTS.—Guinea pigs of either sex (300-800 g) were killed by a blow on the head and exsanguinated. Sections of ileum (4-5 cm) were prepared and mounted in an isolated organ bath (40 ml) bubbled with a 95% O_2-5% CO_2 mixture at 37°. Tyrode's solution was used as the bathing fluid with concentrations in g/liter (mM): 0.2 KCl (2.7); 0.13 $MgSO_4.7H_2O$ (0.53); 0.065 $NaH_2PO_4.2H_2O$ (0.42); 0.27 $CaCl_2.2H_2O$ (1.84); 8.0 NaCl (136); 1.0 $NaHCO_3$ (11.9); 1.0 glucose (5.55) (p.a., Merck). Contractions were measured auxotonically whereby 0.75-1 g tension was applied[2] (4). Concentrations of acetylcholine and histamine (1×10^{-9} g/ml to 1×10^{-4} g/ml) were added in a cumulative way using the method of Van Rossum (5). The segments of ileum were allowed to stabilize with frequent washings for 15-20 min after mounting them in the organ baths. Two control cumulative dose-response curves were done with a 10 min interval and appropriate tissue washing in between. The tested alkaloids were added to the bath 5 min after the last control curve, and after an incubation time of 5 min, another cumulative dose-response curve was made. The strips were then washed, and 10 min later, a last control dose-response curve was done in order to obtain some information about the reversibility of the alkaloid effect studied. The maximum contraction height of the second dose-response curve was considered as a 100% effect, and the results of the other dose-response curves were expressed as a percentage of this maximum contraction. Solvent controls were tested in concurrent experiments utilizing administration of equivalent drug-free solutions.

COAXIAL STIMULATION.—Coaxial stimulation of the guinea-pig ileum was done as described by Laekeman *et al.* (6). After stabilization and taking two cumulative dose-response curves with acetylcholine (1×10^{-9} to 1×10^{-4} g/ml), electrical pulses (40 mA, 0.1 Hz, 1 msec) were given for 15 min. The products were then added to the bath, and tissue stimulation was continued for another 15 min. After the stimulation was stopped, a cumulative dose-response curve with acetylcholine was made. Finally, the ileum was washed, and 10 min later the last dose-response curve was carried out. In this way, the influence of the alkaloids on both exogenously-added and endogenously-released acetylcholine could be evaluated.

In one set of experiments, the reversibility of the alkaloid-induced inhibition of the twitch contractions was studied by using PGE_2 as a physiological agent which has a postsynaptic sensitizing activity (7).

RESULTS AND DISCUSSION

From the cumulative dose response curves a pD_2 of the agonist histamine was calculated: $pD_2=6.13\pm0.05$ (± SEM; n=24), which is in the same order of magnitude as the values found in the literature, 6.6 (5) and 6.09 ± 0.08 (8). The maximal contractions of histamine were depressed in the presence of methuenine (3.4×10^{-5}M). A $pD'_2\pm$SEM could be calculated and amounted to 5.13 ± 0.14 (n=12; Figure 2). 16-Epimethuenine shifted the curves to the right as compared to the control curves (Figure 2). The Schild plot was calculated according to Arunlakshana and Schild (9) and Giudicelli (10) (Figure 3). The $pA_2\pm$SEM was 6.55 ± 0.08 (slope±confidence limits=0.67 ± 0.40, n=7). Methuenine 1×10^{-6}M and 3.4×10^{-6} also depressed the maximum of the dose-response curves with acetylcholine: $pD'_2=5.10\pm0.11$ (± SEM, n=11). Under the same experimental conditions, the mean±SEM pD'_2 obtained for papaverine was 5.08 ± 0.18 (n=11), which can be compared with 4.8 obtained for papaverine in rat intestine (5). The other alkaloids including 16-epimethuenine did not influence the cumulative dose-response curves for acetylcholine in the concentrations used (Figure 4). The pD_2 for acetylcholine (7.20 ± 0.07, ±SEM, n=24) was quite comparable with other authors: 7.49 (9) and 7.17 ± 0.08 (8).

Methuenine (1×10^{-6} and 3.4×10^{-6}M, n=6 for each concentration) and 16-epimethuenine (3.4×10^{-6}M, n=6) significantly inhibited the twitch contractions

[2]Harvard smooth muscle transducers (model 386) were used. Contractions were recorded on a Kipp & Zonen BD9 two-channel recorder.

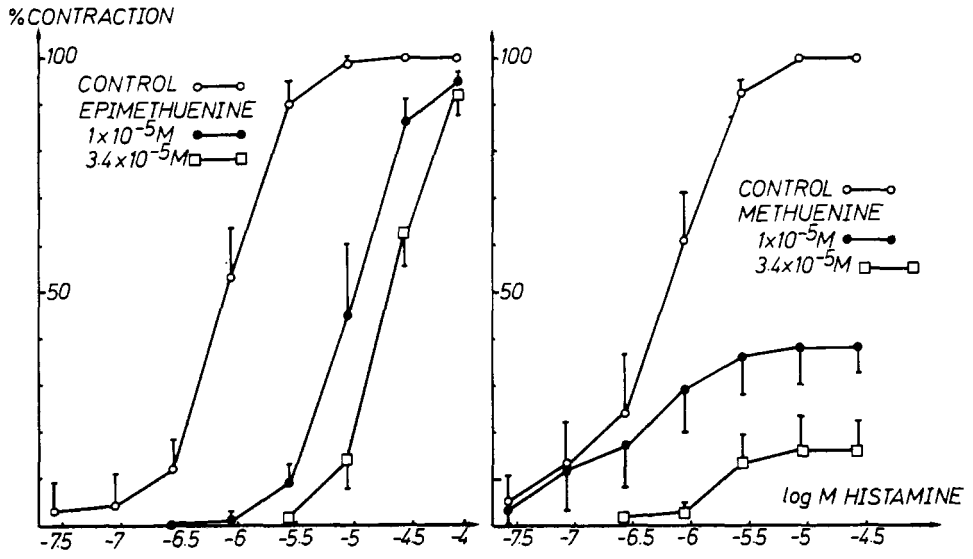


FIGURE 2. Cumulative dose-response curves with histamine: inhibition by 16-epimethuenine and methuenine. Each point represents the mean ± SEM of at least five experiments except for 3.4×10^{-5}M 16-epimethuenine where three experiments were done.

after electrical stimulation, methuenine being the most potent ($p<0.005$ Wilcoxon, Figure 5). 6-Oxomethuenine and methuenine-*N*-oxide showed no inhibitory activity as compared to the control values (3.2×10^{-6}M, n=5 for each product). The inhibitory activity of methuenine could partially be reversed by the addition of PGE_2. After incubation with 3.4×10^{-6}M of methuenine for 15 min, PGE_2 (8.5×10^{-8} and 2.8×10^{-7}M) was able to restore the twitch contractions to 50% or more of the initial level (Figure 6). On the contrary, naloxone (3×10^{-7}M) could not reverse the inhibition induced by methuenine.

Twitch contractions of the guinea-pig ileum elicited by coaxial stimulation, are the result of the post-synaptic activity of acetylcholine which is released in minute quan-

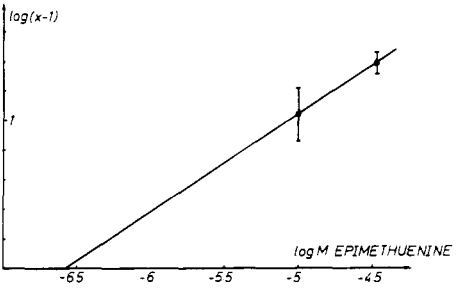


FIGURE 3. Schild plot of the 16-epimethuenine results: competitive antagonism against histamine. Each point represents the mean ± SE of at least three experiments.

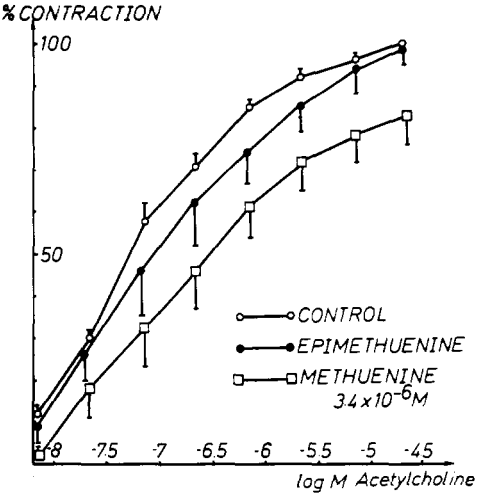


FIGURE 4. Cumulative dose-response curves with acetylcholine: influence of methuenine and 16-epimethuenine. Each point represents the mean ± SEM of six experiments.

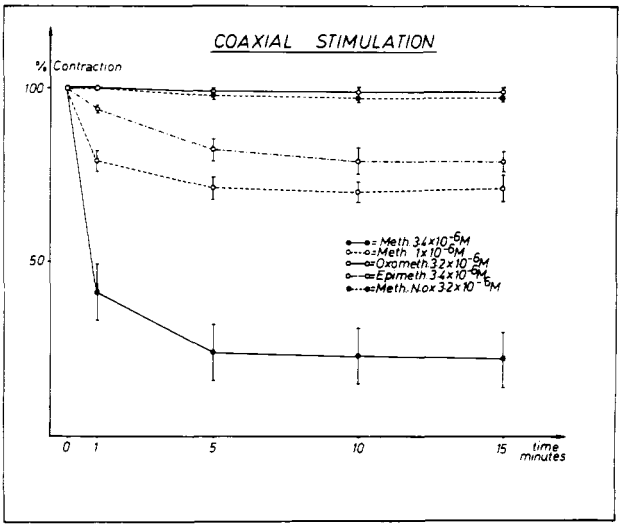


FIGURE 5. Inhibition of coaxial stimulation of the guinea pig ileum by the alkaloids. Each point represents the mean of the value±SEM of at least five experiments. Concentrations are expressed in M (bath concentrations).

tities from the intramural nerve endings during electrical stimulation (11). This experimental set-up allows study of the influence of unknown substances on neurotransmission and on smooth muscle activity. Furthermore, by incorporating cumulative dose-response curves with acetylcholine, the interference of certain drugs with the exogenously added and endogenously-released acetylcholine can be evaluated.

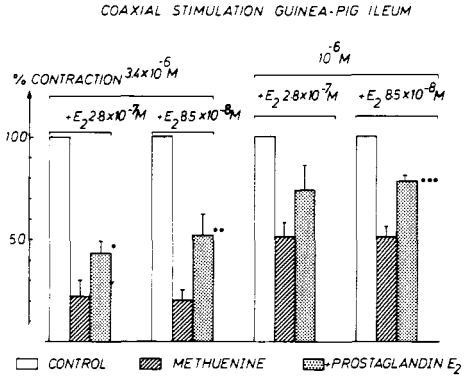


FIGURE 6. Results of the reversal by PGE_2 of inhibition of coaxial stimulation. Each column represents the mean±SEM of at least six experiments. *:$p<0.05$; **:$p<0.01$; ***:$p<0.005$ (Student *t*-test).

Our results show that methuenine inhibited the activity of both exogenously added and endogenously released acetylcholine. Because the inhibition by methuenine could not be reversed by naloxone, the alkaloid is not interfering with the endogenous release of acetylcholine (12). From the experiments with cumulative doses of acetylcholine and histamine, methuenine can be characterized as a non-competitive antagonist, inasmuch as this compound depressed the maximal response of the dose-response curve (5, 13).

The inhibition by methuenine is reversible since PGE_2, given after inhibition of the twitch contractions had occurred, partially restored these contractions. PGE_2 can be considered as a physiological agonist that plays an important role during peristaltic movements in the bowel (14). Furthermore, the cumulative dose-response curves obtained after washing the isolated ileum to remove the alkaloid, returned quickly to the control values. These results clearly show the pharmacological type of inhibition that can be characterized as musculotropic, i.e., similar to the effect of papaverine ("papaverine-like activity"). The pD'_2-values are also quite comparable; however, 16-epimethuenine was not very active in the experiments with coaxial stimulation: 1×10^{-6} g/ml induced only a 20% inhibition after 15 min and did not show a quantitatively evaluable activity in the cumulative dose-response curves with acetylcholine. On the other hand, 16-epimethuenine produced a parallel shift to the right of the dose-response curve of histamine without depressing the maximal contractions. The slope confidence limits included the value of 1.0, and the H_1-antihistaminic potency could be evaluated by pA-calculation.

An interesting point is the difference in pharmacological properties between the two alkaloids. Methuenine and epimethuenine differ only by their configuration at C-16, having, respectively, *cis*- and *trans*- C/D ring junctions (Figure 1). It is suggested by X-ray diffraction studies of the closely related 19-dehydroervatamin molecule (15) that methuenine should contain a normal chair piperidine ring with an axial C-16 proton together with a boat-like seven-membered ring in which C-14 and C-3 are down.

Consequently, the C-15 proton is equatorially-located since nmr studies revealed a *cis*-C/D ring junction for methuenine. In 16-epimethuenine, epimerization most probably has occurred at C-16 because the configuration at C-15 is biogenetically invariant in indole-alkaloids from plants of the Apocynaceae (16). Therefore the C-15 and C-16 protons are transdiaxially located in this molecule.

The influence of chirality on the pharmacological activity pattern of classic antihistaminic compounds (H_1 antagonists) such as diphenhydramine has been demonstrated. A concept of complementation has been applied for the antihistaminic and anticholinergic activities of these compounds depending on the mode of binding of optically active diphenhydramine derivatives to histamine and acetylcholine receptors (17). Thus, a stereoselective interaction of methuenine and 16-epimethuenine with both receptors appears to be reflected by the different pharmacological behaviors of these alkaloids with 16-epimethuenine behaving as a competitive antihistaminic drug and methuenine as a non-competitive antagonist for both acetylcholine and histamine. However, inasmuch as the distance between the centers of the aromatic moiety and the alicyclic N-atom in both alkaloids is not optimal, the competitive histamine antagonist activity of 16-epimethuenine is rather small in comparison with that of commercially available H_1-antihistaminics.

The following pA_2-values were found in the literature: diphenhydramine 7.7 (5); *d*-chloropheniramine 8.0 (9), 9.3 (5); *l*-chlorpheniramine 8.3 (5); mepyramine 9.3 (9); promethazine 8.93 (18). In conclusion, our studies showed that the pharmacological activity seen in screening studies of *P. inconspicua* leaves was due to 2-acylindole alkaloids such as methuenine and 16-epimethuenine. The difference in pharmacological characteristics between the two alkaloids again demonstrates the importance of chirality on the pharmacological activity of drugs.

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