

73. Physovenines: Efficient Synthesis of (–)- and (+)-Physovenine and Synthesis of Carbamate Analogues of (–)-Physovenine. Anticholinesterase Activity and Analgesic Properties of Optically Active Physovenines

by Qian-sheng Yu^{a)}, Chi Liu^{b)}, Margareth Brzostowska^{b)}, Linda Chrisey^{b)}, Arnold Brossi^{b)*}, Nigel H. Greig^{c)}, John R. Atack^{c)}, Timothy T. Soncrant^{c)}, Stanley I. Rapoport^{c)}, and Hans-Eckart Radunz^{d)}

^{a)} Shanghai Institute of Organic Chemistry, Chinese Academy of Science, Shanghai 200032, China

^{b)} Natural Product Section, Laboratory of Structural Biology, NIDDK National Institutes of Health, Bethesda, Maryland 20892, USA

^{c)} Laboratory of Neuroscience, NIA, National Institutes of Health, Bethesda, Maryland 20892, USA

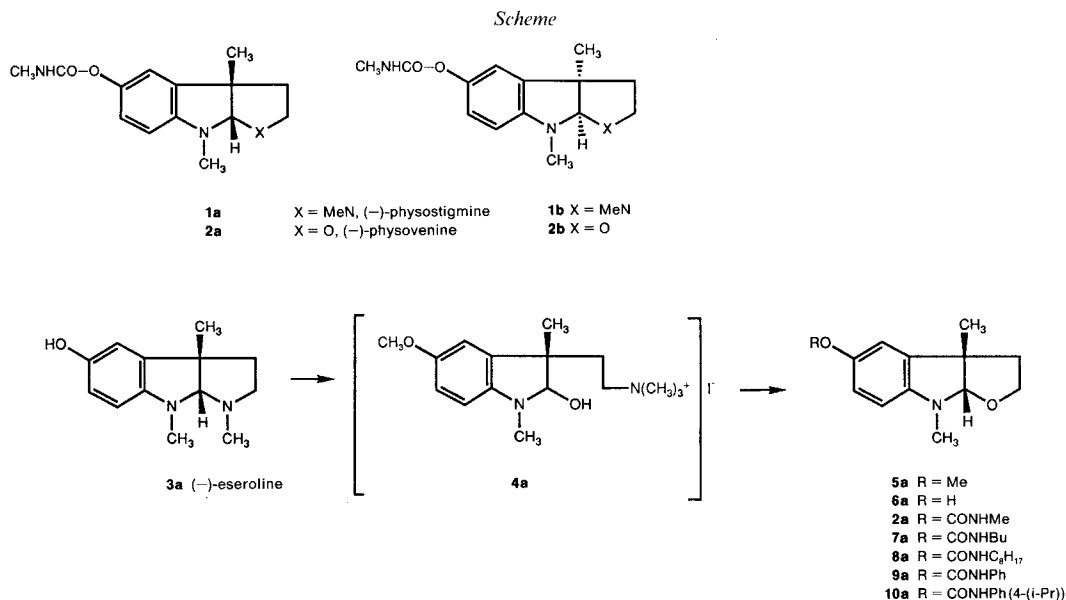
^{d)} Pharmaceutical Research, E. Merck & Co., Inc., D-6100 Darmstadt

Dedicated to Dr. Otto Isler on the occasion of his 80th birthday

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Column chromatography of easily available (±)-physovenine (**2**) on cellulose triacetate afforded (–)- and (+)-physovenine (**2a** and **2b**, resp.). Alkaloids **2a**, **b** required for pharmacological testing were prepared from eserolines (**3a**, **b**) by an improved procedure. Natural (–)-physovenine (**2a**) was equally potent in inhibiting AChE and BChE *in vitro* as natural physostigmine (**1a**), and twice as potent as the unnatural antipode **2b** against AChE and 14 times as potent against BChE. Several carbamate analogs of **2a** were at least as potent as the former compound in these assays. None of the compounds tested did bind to different opiate receptor or serotonin receptor preparations. Most of the compounds tested had considerable analgesic activity in the *Writhing* test.

In search of centrally acting agents potentially useful for treating cholinergic disorders, particularly those manifested by *Alzheimer's* disease, we have embarked on a re-evaluation of the alkaloids from the seeds of *Physostigma venenosum* [1a–e]. Two of the alkaloids, (–)-physostigmine (**1a**; see *Scheme*) and (–)-physovenine (**2a**), were reported to have potent anti-acetylcholinesterase (AChE) activity when assayed *in vitro*, measuring inhibition of AChE obtained from human erythrocytes [2], and **1a** is medically used to reduce intraocular tension in glaucoma, in the treatment of intestinal atony, and in the treatment of urinary retention [3]. Modification of the carbamate group and of the Me–N(1) group in **1a** has afforded several potent analogs [4] [5]. We now have extended this research to another study of the lesser known (–)-physovenine (**2a**) and some of its carbamate-ester analogs and of the unnatural enantiomer (+)-physovenine (**2b**). Natural **2a** was isolated from the basic extracts of *Physostigma venenosum* in 1911 [6] and its structure proposed on the basis of spectral data [7] and proven to be correct by a total synthesis of racemate **2** [8]. The (3*aS*)-configuration of **2a** and the (3*aR*)-configuration of antipode **2b** was elaborated by their enantiospecific synthesis from (–)-physostigmine (**1a**) [9] and (+)-physostigmine (**1a**), respectively [2].



Chemistry. – Although several syntheses of **2** are reported [1d] [10] [11], neither of them seems adaptable to the preparation of its enantiomers. Physovenine (**2**), in contrast to physostigmine (**1**), does not contain a very basic N-atom, and it was considered unlikely that a classical resolution *via* diastereoisomers could be achieved. However, the properties of **2** made it an ideal compound to try its resolution by chromatography on a cellulose-triacetate column, successfully used for separating enantiomeric lactones [12]. Under the elution conditions applied (see *Exper. Part*), this afforded natural (–)-physovenine (**1a**) as the faster eluting material and its (+)-antipode **2b** as the slower eluting fraction. After purification, unnatural **2a** was identical with an analytical standard prepared by partial synthesis, whereas **2b** was found to be less pure.

Having an easy access to optically active eserolines (**3a, b**) by total synthesis [13] [14], we decided, for the preparation of larger quantities of **2a, b**, to follow the route chosen by *Dale* and *Robinson* [2], but to replace eserethole (ethyl ether of eseroline) by the corresponding methyl ether formed *in situ*. Thus, exhaustive methylation of (+)-eseroline (**3a**) with MeI in dimethyl sulfoxide (DMSO) in the presence of powdered KOH, followed by quaternization of the methin bases with MeI, afforded **4a**, which was not isolated but refluxed *in situ* with 7N NaOH to afford physovenol methyl ether (**5a**) in 70% overall yield (see *Scheme*). Conversion of **5a** into physovenol (**6a**) was accomplished with BBr_3 in CH_2Cl_2 followed by decomposition of the boron complexes with MeOH. Reaction of **6a** with methyl isocyanate in dry Et_2O in the presence of a trace of Na afforded natural (–)-physovenine (**2a**), identical in every respect with the alkaloid described [3] [7]. The unnatural antipode **2b** was similarly prepared from (+)-eseroline (**3b**) and was identical with **2a** except for opposite specific rotation [2]. Reaction of **6a** with butyl isocyanate, octyl isocyanate, phenyl isocyanate, and cumyl isocyanate afforded carbamates **7a–10a**, respectively.

Biological Evaluation. – Physostigmine (**1a**) from Calabar beans has provided a valuable tool for elucidating the mechanism of cholinergic transmission and also has been used clinically in the treatment of glaucoma, myasthenia gravis, and, more recently, in *Alzheimer's* disease ([4] and ref. cited therein). The limited efficacy of **1a** in its medical uses may be related to its relatively short half-life.

Anticholinesterase Activity (see Table 1). Natural physovenine (**2a**) was shown to be as potent as natural (–)-physostigmine (**1a**) in assays measuring inhibition of acetylcholinesterase (AChE) from human erythrocytes, whereas antipode **2b** was practically inactive [2]. We now have repeated these experiments with **2**, **2a**, and **2b** in assays

Table 1. IC_{50} Values of Physovenines and Several Carbamate Analogues vs. Human Erythrocyte AChE and Human Plasma BChE^{a)}^{b)}

	IC_{50} [nmol]	
	AChE	BChE
(–)-Physostigmine (1a)	27.9± 2.4	16.0± 2.9
(±)-Physovenine (2)	29.6± 11.2	4.2± 1.4
(–)-Physovenine (2a)	27.1± 0.8	3.7± 1.4
(+)-Physovenine (2b)	55.7± 1.3	55.9±14.6
(±)-7-Bromophysovenine ^{c)}	62.1± 18.3	3.8± 0.9
(–)-Butylcarbamate 7a	19.8± 1.7	1.4± 0.2
(–)-Octylcarbamate 8a	25.8± 1.2	10.6± 3.0
(–)-Phenylcarbamate 9a	11.2± 2.1	700.0±37.0
(–)-Cumylcarbamate 10a	3859.9±970.9	16.5± 2.2

^{a)} As described in [4]. ^{b)} Values are mean ±s.d. of 2–4 separate assays. ^{c)} Prepared according to [10].

measuring inhibition of AChE and of butylcholinesterase (BChE) from human plasma by procedures developed earlier [4]. Included in the experiments were carbamates **7a**–**10a**, and the results are summarized in Table 1. The almost identical potencies of **1a** and **2a** in inhibiting AChE and BChE *in vitro* show that a basic N-atom (N(1)) in the tricyclic molecule need not be present, and that interaction with the enzyme at this point is mediated through a H-bond. The high activity of **2a** against AChE and BChE is in good support of the view that the central effects observed with these compounds are caused by tricyclic molecules and not ring-open indolium species [15]. Good activity against both enzymes, AChE and BChE, is shown in these assays by carbamates **7a** and **8a**. The phenylcarbamate **9a** is remarkably potent against AChE and considerably less potent against BChE. The cumylcarbamate **10a** which is substituted with an i-Pr group at C(4) of the phenyl ring, however, shows the opposite behavior.

Analgesic Activity (see Table 2). Reports on analgesic activity of (–)-physostigmine (**1a**) [16], possibly related to the opiate-like effects of its metabolite (–)-cseroline [17], suggest that some of the compounds prepared be assayed for binding to different opiate receptors *in vitro* and for *in vivo* activity in the *Writhing* test. It can be seen from Table 2 that these compounds do not exhibit opiate-like analgesic effects and do not bind to the 5-HT_{1A} receptor, but show considerable activity in the *Writhing* test. The natural alkaloid **2a** is twice as potent as the unnatural isomer **2b**. Unfortunately, the compounds

Table 2. Binding (IC_{50}) and Analgesic Activity (ED_{50}) of (–)- and (+)-Physovenine, (±)-Physovenol, and (±)-Bromophysovenine^{a)}

	IC_{50} [nmol/l]				ED_{50} [mg/kg]
	μ -Receptor ^{b)}	δ -Receptor ^{c)}	κ -Receptor ^{d)}	5-HT _{1A} ^{e)}	Writhing test ^{f)} <i>s.c.</i>
(+)-Physovenine (2b)	$> 1 \cdot 10^{-5}$	$> 1 \cdot 10^{-5}$	$> 1 \cdot 10^{-6}$	$> 1 \cdot 10^{-6}$	0.4
(–)-Physovenine (2a)	$> 1 \cdot 10^{-5}$	$> 1 \cdot 10^{-5}$	$> 1 \cdot 10^{-6}$	$> 1 \cdot 10^{-6}$	0.2
(±)-Physovenol (3)	$> 1 \cdot 10^{-5}$	$> 1 \cdot 10^{-5}$	$1 \cdot 10^{-6}$	n.d.	3.4
(±)-7-Bromophysovenine ^{b)}	$> 1 \cdot 10^{-5}$	$> 1 \cdot 10^{-5}$	$> 1 \cdot 10^{-5}$	$> 1 \cdot 10^{-6}$	0.6
(–)-Phenylcarbamate 9a	$1 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$1.6 \cdot 10^{-7}$	n.d.	ⁱ⁾

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^{b)} Rat brain, ligand PL-0.17 [18].

^{c)} Rat brain, ligand DPDPE [18].

^{d)} Guinea-pig cerebellum, ligand U-69593 [19].

^{e)} Rat hippocampus, ligand 8-OH-DPAT [20].

^{f)} Mice [21].

^{g)} Morphine: 0.4.

^{h)} See [10].

ⁱ⁾ No significant effect up to 1 mg.

show significant toxicity after *s.c.* application to mice, making this group of compounds unattractive for further development as analgesic agents.

Experimental Part

General. TLC: silica-gel plates, CH_2Cl_2 /MeOH 100:1, unless stated otherwise. M.p.: in capillary tubes; uncorrected. Optical rotations: Perkin-Elmer-241-MC polarimeter (25°). ¹H-NMR spectra: EM-306L (60 MHz) spectrometer; chemical shifts are reported in δ units with TMS as internal standard. Mass spectra: Finnigan-4021 instrument. Elemental analysis were performed by the Shanghai Institute of Organic Chemistry.

(–)-Eseroline (**3a**). From (–)-physostigmine (**1a**) as published in [13].

(–)-Physovenol Methyl Ether (= (3*aS*,8*aS*)-3,3*a*,8,8*a*-Tetrahydro-5-methoxy-3*a*,8-dimethyl-2H-furo[2,3-*b*]indol; **5a**). To (–)-eseroline (**3a**; 620 mg, 2.83 mmol); in DMSO (5 ml) under N_2 , powdered KOH (634 mg, 11.3 mmol) was added. After stirring for 5 min at r.t. under N_2 , MeI (803 mg, 5.66 mmol) was added and stirring continued for 1 h. Then, MeI (1606 mg, 11.32 mmol) was added and the mixture stirred for another h. The mixture was washed with Et_2O (2 $\times$ 50 ml) to remove excess MeI and DMSO, and the remaining slurry evaporated *in vacuo* to remove low-boiling solvents. Then 7*N* NaOH (18 ml) was added and the mixture refluxed for 6 h. After cooling, the soln. was extracted with Et_2O (3 $\times$ 20 ml) and the combined extract washed with brine (10 ml), dried ($MgSO_4$), and evaporated; **5a** as an oil (485 mg, 78.3%). $[\alpha]_D = -80.3$ ($c = 0.6$, EtOH). ¹H-NMR and MS: identical with those reported for the racemic compound [10].

(–)-Physovenol (= (3*aS*,8*aS*)-3,3*a*,8,8*a*-Tetrahydro-3*a*,8-dimethyl-2H-furo[2,3-*b*]indol-5-ol; **6a**). To **5a** (760 mg, 3.23 mmol) in CH_2Cl_2 (30 ml), BBr_3 (2.5 ml) in CH_2Cl_2 (10 ml) was added dropwise with stirring under N_2 at r.t. After 2 h, MeOH (5 ml) was added dropwise under exterior cooling, and volatile gases were released by opening of the vessel. After evaporation, the residue was dissolved in H_2O (20 ml) and the soln. made alkaline by addition of an aq. $NaHCO_3$ soln. After extraction with Et_2O (3 $\times$ 30 ml), the org. layer was washed with brine (20 ml), dried ($MgSO_4$) and evaporated; **6a** as yellowish crystals (643 mg, 97%). M.p. 144–146° (C_6H_6). $[\alpha]_D = -93.8$ ($c = 0.6$, $CHCl_3$). ¹H-NMR and MS: identical with those reported for the racemic compound [10].

(–)-5-O-(Octylcarbamoyl)physovenol (= (3*aS*,8*aS*)-3,3*a*,8,8*a*-Tetrahydro-3*a*,8-dimethyl-2H-furo[2,3-*b*]indol-5-yl N-Octylcarbamate; **8a**). To a soln. of **6a** (100 mg, 0.48 mmol) in anhyd. Et_2O (10 ml), a small piece of Na (*ca.*

1 mg) was added. After stirring for 5 min at r.t. under N_2 , octyl isocyanate (90 mg, 0.58 mmol) was added dropwise. After the addition, the mixture was stirred for 1 h at r.t., the solvent evaporated, and the residue flash-chromatographed (silica gel, $CH_2Cl_2/MeOH$ 20:1): **8a** as an oil (125 mg, 71.4%). TLC: uniform. $[\alpha]_D = -71.2$ ($c = 0.5$, EtOH). MS: 361 ($[M + 1]^+$).

(–)-*Physovenine* (= (3*aS*,8*aS*)-3,3*a*,8,8*a*-Tetrahydro-3*a*,8-dimethyl-2H-furo[2,3-*b*]indol-5-yl N-Methylcarbamate; **2a**). Similarly prepared from **6a** and methyl isocyanate (AcOEt). Yield 79%. M.p. 125–126° (AcOEt) ($[16]$: m.p. 124–125°). $[\alpha]_D = -92$ ($c = 0.1$, EtOH; $[16]$: $[\alpha]_D = -92$ (EtOH)). MS: 263 ($[M + 1]^+$).

(–)-5-O-(Butylcarbamoyl)physovenol (= (3*aS*,8*aS*)-3,3*a*,8,8*a*-Tetrahydro-3*a*,8-dimethyl-2H-furo[2,3-*b*]indol-5-yl N-Butylcarbamate; **7a**). Similarly prepared from **6a** and butyl isocyanate. Oil (91%). $[\alpha]_D = -71.4$ ($c = 0.5$, EtOH). MS: 305 ($[M + 1]^+$).

(–)-5-O-(Phenylcarbamoyl)physovenol (= (3*aS*,8*aS*)-3,3*a*,8,8*a*-Tetrahydro-3*a*,8-dimethyl-2H-furo[2,3-*b*]indol-5-yl N-Phenylcarbamate; **9a**). Similarly prepared from **6a** with phenyl isocyanate. Yield 80.6%. M.p. 126–127° (AcOEt). $[\alpha]_D = -60.7$ ($c = 0.6$, $CHCl_3$). 1H -NMR ($CDCl_3$): 1.40 (s, Me–C(3*a*)); 1.90–1.95 (m, CH_2 (3)); 2.90 (s, Me–N(8)); 3.00–4.00 (m, CH_2 (2)); 4.93 (s, H–C(8*a*)). MS: 324 (M^+). Anal. calc. for $C_{19}H_{20}N_2O_3$ (324.26): C 70.35, H 6.21, N 8.64; found: C 70.30, H 6.22, N 8.61.

(–)-5-O-(Cumylcarbamoyl)physovenol (= (3*aS*,8*aS*)-3,3*a*,8,8*a*-Tetrahydro-3*a*,8-dimethyl-2H-furo[2,3-*b*]indol-5-yl N-(4-Isopropylphenyl)carbamate; **10a**). Similarly prepared from **6a** and 4-isopropylphenyl isocyanate (= cumyl isocyanate). Yield 63%. M.p. 167–169° (AcOEt). $[\alpha]_D = -54.6$ ($c = 0.5$, EtOH). MS: 367.4 ($[M + 1]^+$).

Unnatural (+)-Series. The unnatural (+)-series of compounds was similarly prepared from (+)-physovenol (**6b**), prepared from (+)-eseroline (**3b**) [13]. The (+)-enantiomers gave TLC, 1H -NMR, and MS identical to those obtained in the (–)-series. The following compounds were prepared:

(+)-Physovenol Methyl Ether (**5b**). Oil. $[\alpha]_D = +81.10$ ($c = 0.5$, EtOH).

(+)-Physovenol (**6b**). Brownish crystals. M.p. 144–146° (C_6H_6). $[\alpha]_D = +91$ ($c = 0.5$, $CHCl_3$).

(+)-Physovenine (**2b**). Yellowish crystals. M.p. 122–124° (AcOEt; $[2]$: m.p. 120–122°). $[\alpha]_D = +92$ ($c = 0.1$, EtOH).

Chromatographic Separation of (±)-Physovenine (**2**) into Enantiomers. A class column (30 cm, i.d. 2.5 cm) was slurry packaged with 40 g of cellulose triacetate (Merck, art. No. 16362) which was swollen before in 100 ml of 95% EtOH at 75° for 20 min. After removal of excess solvent, the stationary phase was washed (95% EtOH, 50 ml). (±)-Physovenine (**2**, 50 mg), prepared by the published procedure [10], was dissolved in the eluent (0.5 ml, 95% EtOH), and 51 fractions of 3 ml were collected (flow rate 0.5 ml/min). Fractions 1–25 which showed a negative $[\alpha]$ were combined and chromatographed through a small column (silica gel, $CH_2Cl_2/MeOH$ 200:1), affording, after evaporation and crystallization from AcOEt, TLC-pure **2a** (10 mg). M.p. 127–128°. $[\alpha]_D = -73.8$ ($c = 0.5$, EtOH).

Enantiomer **2b** was obtained from Fractions 43–51 which showed a positive $[\alpha]$. Similar treatment as described for **2a** afforded **2b** (9.5 mg), identical with the material prepared before. M.p. 127–128°. $[\alpha]_D = +93.5$ ($c = 0.5$, EtOH).

Biological Experiments. Anti-Cholinesterase Activity. AChE and BChE inhibition of compounds was determined against human erythrocyte AChE and plasma BChE in 0.1M Na_3PO_4 buffer (pH 8.0), using the spectrophotometric method of Ellman *et al.* [22]. Freshly collected blood was centrifuged ($6000 \times g$, 10 min, 4°) and the plasma removed and diluted 1:125 with 0.1M Na_3PO_4 (pH 7.4). The erythrocytes were washed 3 times in isotonic saline, lysed in 9 volumes of 0.1M Na_3PO_4 containing 0.5% Triton-X (Sigma Chemical Co., St. Louis, MO), and diluted with 19 volumes of 0.1M Na_3PO_4 (final dilution 1:200). Acetyl-β-methylthiocholine (0.5 mM; Sigma) and *s*-butyrylthiocholine (0.5 mM; Sigma) were used as specific substrates for the assay of AChE and BChE, respectively [4]. For each cholinesterase preparation, 25 μl of substrate and enzyme was added to a final incubation volume of 0.75 ml.

Compounds were dissolved in Tween 80/EtOH 3:1 (*v:v*; 150 μl total volume), diluted in half log-intervals to provide a range of final incubation concentrations between $1 \cdot 10^{-5}$ M and $3 \cdot 10^{-10}$ M and preincubated with the enzymes (30 min at 21°) prior to addition of the substrates. The Tween 80/EtOH was diluted in excess of 1:3000 and did not affect either AChE or BChE activity. Following 25 min incubation at 37°, production of a yellow thionitrobenzoate anion was measured with a spectrophotometer set at $\lambda = 412$ nm. To correct for nonspecific substrate hydrolysis, aliquots of AChE and BChE were incubated under conditions of complete enzyme inhibition (by the addition of $1 \cdot 10^{-5}$ M physostigmine), and the change in absorbance under this condition was subtracted from that observed with varying concentrations of the test compounds. Further, the AChE and BChE activity of each compound was determined with physostigmine as control and an external standard, whose activity we have previously reported [4].

The enzyme activity of each concentration was expressed as a percentage of the activity determined in the absence of inhibitor, transformed to a logit format (logit = % activity/100 – % activity) and then plotted as a

function of the log concentration of the compound. An IC_{50} , defined as the concentration in nmol required to inhibit 50% of enzyme activity (logit = 0; i.e., logit = $[50/100-50]$), was then determined. In each case, an IC_{50} was determined only when more than 50% inhibition was achieved from duplicate samples analyzed on several occasions.

Analgesic Activity. All the information pertinent to measuring these effects is mentioned in Table 2.

REFERENCES

- [1] a) L. Marion, *Alkaloids* **1952**, 2, 438; b) E. Coxworth, *ibid.* **1965**, 8, 27; c) B. Robinson, *ibid.* **1967**, 10, 383; d) B. Robinson, *ibid.* **1971**, 13, 213; e) S. Takano, K. Ogasawara, *ibid.* **1989**, 36, 225.
- [2] F. J. Dale, B. Robinson, *J. Pharm. Pharmacol.* **1970**, 22, 889.
- [3] 'The Merck Index', 11th edn., Merck & Co., Rahway, N.J., USA, 1989, Index-No. 7357.
- [4] J. R. Attack, Q. S. Yu, T. T. Soncrant, A. Brossi, S. I. Rapoport, *J. Pharm. Exp. Ther.* **1989**, 249, 294.
- [5] A. Brossi, *J. Med. Chem.* **1990**, 33, 2311.
- [6] A. H. Salway, *J. Chem. Soc.* **1911**, 99, 21148.
- [7] B. Robinson, *J. Chem. Soc.* **1964**, 5103.
- [8] R. B. Longmore, B. Robinson, *Collect. Czech. Chem. Commun.* **1967**, 32, 2184.
- [9] R. B. Longmore, B. Robinson, *Chem. Ind.* **1966**, 1638.
- [10] Y. Luo, Q. S. Yu, L. Chrisey, A. Brossi, *Heterocycles* **1990**, 31, 283.
- [11] K. Shishido, T. Azum, M. Shibuya, *Heterocycles* **1990**, 31, 219.
- [12] E. Francotte, D. Lohman, *Helv. Chim. Acta* **1987**, 70, 1569.
- [13] Q. S. Yu, B. Schönenberger, A. Brossi, *Heterocycles* **1987**, 26, 1273.
- [14] Q. S. Yu, A. Brossi, *Heterocycles* **1988**, 27, 747.
- [15] A. Brossi, *J. Nat. Prod.* **1985**, 48, 878.
- [16] W. A. Klee, R. A. Streaty, *Nature (London)* **1974**, 248, 61.
- [17] B. Schönenberger, A. E. Jacobson, R. Streaty, W. A. Klee, J. L. Flippen-Anderson, R. Gilardi, *J. Med. Chem.* **1986**, 29, 2268.
- [18] P. L. Wood, S. E. Carlson, D. Lane, R. L. Hudgin, *Neuropharmacology* **1981**, 20, 1215.
- [19] R. A. Lathi, M. M. Mickelson, J. M. McCall, D. F. Voigt-Lander, *Eur. J. Pharmacol.* **1985**, 109, 281.
- [20] J. M. Cossery, *Eur. J. Pharmacol.* **1987**, 140, 143.
- [21] a) L. C. Hendershot, J. Forsaith, *J. Pharmacol. Exp. Ther.* **1959**, 125, 237; b) E. Siegmund, R. Cadmaus, Go Lu, *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol.* **1964**, 247, 729.
- [22] G. L. Ellman, K. D. Courtney, V. Anders, Jr., R. M. Featherstone, *Biochem. Pharmacol.* **1961**, 7, 86.