to Dr. C. A. de Bock and Dr. W. T. Goedemans for supplying and discussing the biological data and to colleagues and coworkers of the analytical department, especially to Miss M. E. van der Heeden, for measurements and interpretation of the spectra.

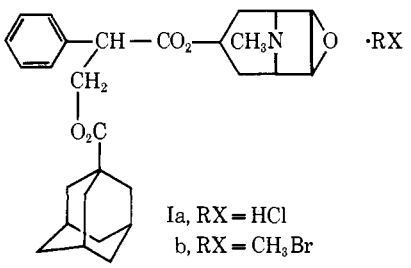
1-Adamantanecarboxylic Acid Ester of Scopolamine

ROBERT BRUCE MOFFETT

Research Laboratories of The Upjohn Company, Kalamazoo, Michigan 49001

Received March 27, 1969

A few years ago it was found[1] that the esters of scopolamine were powerful anticholinergics. One of these, scopolamine pivalate hydrochloride, was found to be very effective as a topical antiperspirant.[2] In order to assess the influence of the rigid structure the 1-adamantanecarboxylic acid ester of scopolamine (I) has now been made.



Ia, RX = HCl
b, RX = CH_3Br

Both the hydrochloride (Ia) and the methobromide (Ib) were found to be powerful anticholinergics. They markedly dilated mouse pupils when injected intraperitoneally at doses of 20% of their LD_{50}'s. Ia had an LD_{50} of 562 mg/kg and Ib, 56 mg/kg.[3] Further testing is desirable to determine their minimum effective doses and to evaluate the degree of usefulness of Ia as an antiperspirant.

Experimental Section[4]

1-Adamantanecarboxylic Acid Ester of Scopolamine Hydrochloride (Ia).—1-Adamantanecarbonyl chloride was prepared from 12.6 g (0.07 mole) of the acid and 30 ml of $SOCl_2$. After removing the excess $SOCl_2$ under reduced pressure and purging with C_6H_6, the crude acid chloride was dissolved in 10 ml of C_6H_6 and added to a suspension of 19.22 g (0.05 mole) of dried scopolamine hydrobromide in 50 ml of dry pyridine under N_2. The solid was dissolved by warming and the mixture was allowed to stand overnight at room temperature. The mixture was basified with aqueous Na_2CO_3 and extracted (Et_2O). The Et_2O solution was washed (H_2O, saturated NaCl), dried (Na_2SO_4), and evaporated under reduced pressure. The gummy free base was dissolved in 500 ml of absolute Et_2O and acidified with ethereal HCl giving 27.5 g of white solid hydrochloride. Recrystallization from 150 ml of *i*-PrOH yielded 19.6 g (78%) of white crystals, mp 218–221° dec. *Anal.* ($C_{28}H_{36}ClNO_5$) C, H, Cl, N.

The Methobromide Ib.—Free base was liberated from 5.02 g (0.01 mole) of the above hydrochloride with Na_2CO_3 and extracted (Et_2O). The Et_2O solution was washed (H_2O, saturated NaCl) and evaporated under reduced pressure. To the gummy free base in 25 ml of cold EtCOMe was added 10 ml of cold MeBr. The flask was stoppered, clamped and allowed to stand at room temperature for 3 days. The quaternary salt was collected, washed (EtCOMe and Et_2O), and dried yielding 5.5 g (98%) of white crystals, mp 226.5–227.5° dec. *Anal.* ($C_{29}H_{38}BrNO_5$) C, H, Br, N.

(1) R. B. Moffett and B. D. Aspergren, *J. Amer. Chem. Soc.*, **78**, 3448 (1956).

(2) F. S. K. MacMillan, H. H. Reller, and F. H. Synder, *J. Invest. Dermatol.*, **43**, 363 (1964).

(3) For the method see R. B. Moffett, A. R. Hanze, and P. H. Seay, *J. Med. Chem.*, **7**, 178 (1964), Table I, footnote *a*.

(4) Melting points were taken in capillary tubes with a partial immersion thermometer. Calibration of the apparatus against standard compounds showed no need for correction. Ir spectra were obtained on both compounds and nmr on the hydrochloride. These were in accordance with the proposed structures. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for these elements or functions were within ±0.4% of theoretical.

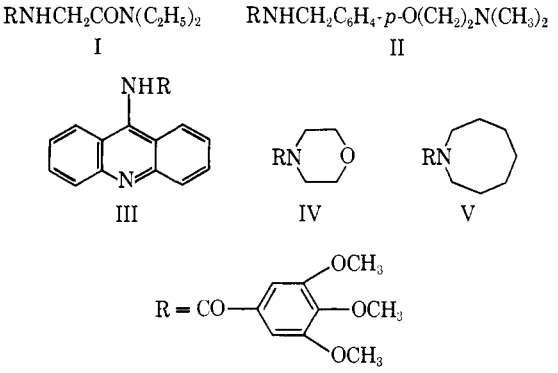
Structure-Activity Studies of 3,4,5-Trimethoxybenzamides. I. Variation of the Amine Function

W. A. SKINNER, J. KENNEDY, J. DEGRAW, AND H. JOHNSON

Department of Pharmaceutical Chemistry, Stanford Research Institute, Menlo Park, California 94025

Received February 12, 1969

Various types of biological activity have been reported for 3,4,5-trimethoxybenzamides. The simplest member of the series, 3,4,5-trimethoxybenzamide, was found to have hypotensive effects and to potentiate the effects of phenobarbital.[1] Trimeglamide[2] (I) has been reported to possess hypnotic activity. Tigan (II) is well known as an antiemetic. Compound III has been reported[3] to have hypotensive activity and to potentiate muscle contraction. Vargha, *et al.*,[4] have synthesized a series of trimethoxybenzamides. A study[5] on one of these, N-(3,4,5-trimethoxybenzoyl)tetrahydro-1,4-oxazine (IV), has shown it to possess interesting tranquilizing properties. Compound V, however, reportedly possesses antidepressant activity.[6] The effect of IV on spontaneous activity of mice was compared with its effect on muscle function using the rotarod.[5] A comparison of the effective dose for depression of activity to that required for rotarod effects gives a measure of the selectivity of drug action. One would want to



(1) P. C. Dandiya, P. K. Sharma, and M. K. Menon, *Indian J. Med. Res.*, **50**, 750 (1962).

(2) G. Cronheim, J. T. Gourzis, and I. M. Tockes, *Science*, **128**, 1570 (1958).

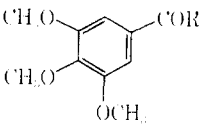
(3) A. Pimenta, J. E. Murad, and D. P. Lenza, *Therapie*, **17**, 1189 (1962).

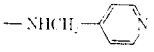
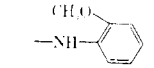
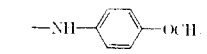
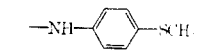
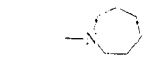
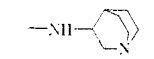
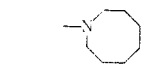
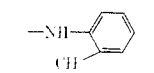
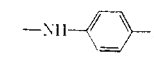
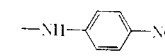
(4) J. Borsy, B. Dumbovich, L. Vargha, and L. Farkas, Hungarian Patent 147,687 (1960); *Chem. Abstr.*, **58**, 7950 (1963).

(5) J. Borsy, *Arch. Intern. Pharmacodyn.*, **126**, 426 (1960).

(6) M. Gibaldi, S. Feldman, and T. R. Bates, *J. Pharm. Sci.*, **57**, 709 (1968).

TABLE I
3,4,5-TRIMETHOXYBENZAMIDES



No.	R	Crystn solvent	Mp, °C	Yield, %	Formula[a]
1		C_6H_6–EtOH	159–162	76	$C_{16}H_{18}N_2O_4$
2		C_6H_6–C_6H_{12}	115–116	76	$C_{17}H_{19}NO_5$
3		C_6H_6–C_6H_{12}	158.5–159	84	$C_{17}H_{19}NO_5$
4		[a]	67–68	70	$C_{16}H_{23}NO_4$
5		C_6H_6–C_6H_{12}	114–118	78	$C_{17}H_{19}NO_4S$
6		C_6H_{12}	71.5–72.5	30	$C_{16}H_{23}NO_4$
7		C_6H_6–EtOH	193–195	64	$C_{17}H_{24}N_2O_4$
8		C_6H_{12}	80–82[b]	84	$C_{17}H_{25}NO_4$
9		C_6H_6–C_6H_{12}	134.5–135	78	$C_{17}H_{19}NO_4$
10		C_6H_6	170–171.5	98	$C_{16}H_{16}FNO_4$
11		C_6H_6–EtOH	192–193	83	$C_{16}H_{16}N_2O_6$

[a] Syrup crystallized on standing. [b] H. A. Luts, W. A. Zucarello, W. L. Nobles, and J. F. Grattan, *J. Pharm. Sci.*, **55**, 1459 (1966). All compounds analyzed within accepted limits for C, H, N.

divorce sedative from coordinated muscle effects for an ideal tranquilizer. In attempts to achieve such separation of pharmacological actions and to define structure–activity relationships pertinent to compounds such as IV and V, we have synthesized a series of 3,4,5-trimethoxybenzamides in which the amine function was varied in terms of both electronic and stereochemical characteristics.

Pharmacology.—Compounds were subjected to pharmacological examination by a preliminary screening procedure intended to differentiate both degree and type of CNS depression. Dose–response relationships were determined for effects on both uncoordinated and coordinated motor activity (Figure 1). With the exception of **9** all compounds exhibited moderate to marked sedative effects at doses below 100 mg/kg as measured by the activity cage method. The compounds were more effective than meprobamate but much less potent than haloperidol. The degree of sedation attainable with increased dosage appeared to be quite limited in the case of compounds **2** and **6** and reversible in **7** and **10** as in meprobamate. None of the benzamides exhibited muscle relaxant activity comparable to meprobamate; all of those tested exhibited shallow dose-response curves for impairment of coordinated motor activity (rotarod) and steeper dose–response curves for depression of uncoordinated motor activity (photocell). Thus, in comparison with meprobamate most of the benzamides exhibited moderate sedative activity with minimal impairment of coordinated motor activity and their activity profiles were qualitatively similar to that of haloperidol. In terms of selective tranquilizing activity some of these trimethoxybenzamides appeared to be more effective than trimethoxybenzoylmorpholine (IV) whose pharmacology was examined in detail by Borsy.[5]

From a structure-activity standpoint the most striking finding was the relative insensitivity of both coordinated and uncoordinated motor depressant activity to structural change at the amide nitrogen. It appears, however, that in the aromatic amine derivatives the presence of an *ortho* substituent was deleterious in terms of uncoordinated motor depressant activity (**2** and **9**). Of further interest was the contrast in properties of the penta-, hexa-, and heptamethyleneimine derivatives (**4**, **6**, **8**). Only **8** failed to exhibit significant depressant activity at 25 mg/kg. More detailed studies[6] have shown that this compound is pharmacologically similar to the tricyclic antidepressants in terms of actions elicited at low dosage levels. The present results, however, emphasize the pharmacological similarity of this compound to other members of the series at higher dosage levels.

That effects on coordinated motor activity are independent of sedative activity in these compounds is suggested by the contrasts in dose–response relationships and effects of structural change on the two actions. It is apparent, also, that variations in the amine function can alter the depressant–antidepressant activity spectrum from a dose–effect standpoint with relatively little effect on the degree of impairment of coordinated motor activity.

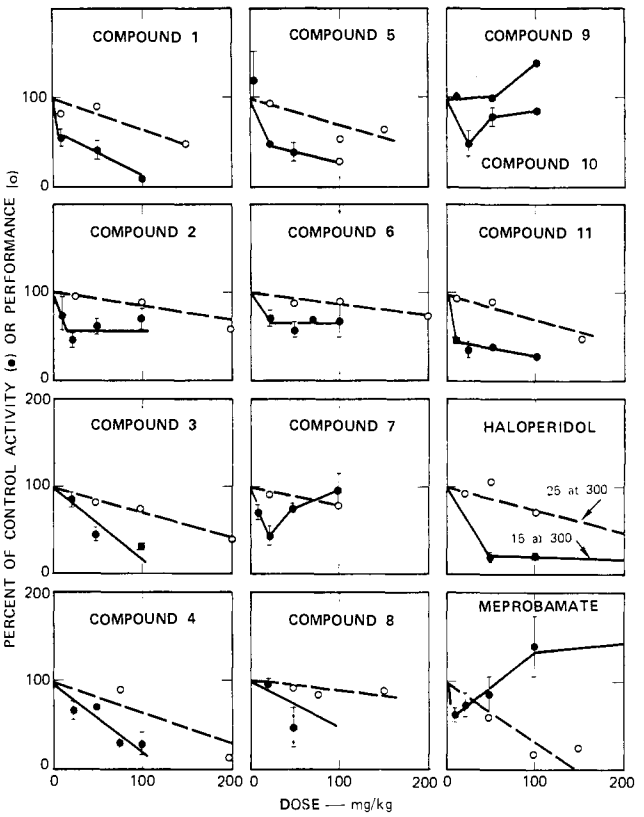


Figure 1.—Effects of trimethoxybenzamides on motor activity of mice as measured by photocell activity cage and rotarod methods. Dose–response data are expressed as per cent of control activity (photocell). Bars represent ± standard errors for means of three to four determinations; all other points are means of two determinations (groups of five mice). Open circles (rotarod) are means of ten animals. Note that doses of haloperidol are scale values $\times 10^{-2}$, *i.e.*, 0.5, 1.0, and 3.0 mg/kg. Rotarod data were not obtained for compounds **9** and **10**.

Experimental Section

Melting points were taken on a Fisher-Johns apparatus and are corrected. Elemental analyses were within ±0.4% of theoretical values.

Pharmacological Methods.—A photocell–activity cage–rotarod screening procedure similar to that described by Kinnard and Carr[7] was used. Male, albino mice (17–20 g) of a Swiss–Webster strain were utilized, and were used only once. All drugs were administered intraperitoneally as suspensions in 10% Tween 80 in 0.9% saline (0.2 ml). Photocell activity was determined as cumulative counts over a 1-hr period beginning 0.5 hr after administration of drug or vehicle to groups of five mice. Activity was determined for one control group with each two treatment groups. Values for the latter were calculated as percentages of the former. Rotarod performance times were determined in groups of five trained mice 0.5 hr after administration of drug or vehicle. Mean performance time for control groups was 113.9 sec. Rod rotation was at the rate of 15 rpm.

Synthesis.—The amines in benzene were refluxed for 2–5 hr with 3,4,5-trimethoxybenzoyl chloride in a 2.2:1 ratio. The precipitated product was either isolated directly or the solution was washed with 3 *N* HCl and a saturated $NaHCO_3$ solution. The C_6H_6 layer was dried ($MgSO_4$) and evaporated *in vacuo*. The products were recrystallized using the solvents listed in Table I.

Acknowledgment.—The authors are grateful to Mrs. A. W. Burfine for technical assistance in the pharmacological evaluations. This work was supported by Stanford Research Institute Research and Development Program.

(7) W. J. Kinnard, Jr., and C. J. Carr, *J. Pharmacol. Exptl. Therap.*, **121**, 354 (1957).

Purine N-Oxides. XXIX. The Synthesis of 6-Hydroxylaminopurine 3-Oxide and Related Derivatives[1]

ALFREDO GINER-SOROLLA

Division of Cell Biochemistry, Sloan-Kettering Institute for Cancer Research, New York, New York 10021

Received March 21, 1969

6-Hydroxylaminopurine,[2] an analog of adenine and hypoxanthine, was found to possess a growth-inhibitory activity against Sarcoma 180 in tissue culture,[3] and inhibited the growth of Ehrlich ascites carcinoma in mice.[4] It was suggested that 6-hydroxylaminopurine may act as an antagonist of adenine and hypoxanthine metabolism and as an inhibitor of purine nucleotide biosynthesis.[4] Studies of the inhibitory effects of 6-hydroxylaminopurine on Ehrlich ascites cells[5] showed that the drug blocked the conversion of inosinate to adenylate and guanylate, thus leading to a decrease in the biosynthesis of nucleic acids.

6-Hydroxylaminopurine was toxic[2b,c] when administered to animals mainly because it was metabolized to the sparingly soluble 2,8-dihydroxyadenine which deposited in the kidney tubules in crystalline form.[6]

The toxicity of biologically active purines may be decreased by their conversion to an N-oxide.[7] It was therefore desirable to synthesize 6-hydroxylaminopurine N-oxide in order to learn whether the toxicity of 6-hydroxylaminopurine[2b,c] could be decreased with retention of its growth-inhibitory activity.

It will also be of interest to see whether 6-hydroxylaminopurine 3-oxide would exhibit a cancerogenic activity, a property shown by a variety of purine N-oxides (especially the 3-oxides)[8] and by the potent oncogenic structural analog 4-hydroxylaminoquinoline 1-oxide.[9]

The parent compound, 6-hydroxylaminopurine, is not significantly oncogenic when administered for prolonged periods to rats,[10] but it has shown mutagenic

(1) This investigation was supported by funds from the National Cancer Institute (Grant No. CA 08748), The Atomic Energy Commission (Contract No. AT[30-1], 910), and aided by the Grant No. T-128F from the American Cancer Society.

(2) (a) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 392 (1958); (b) A. Giner-Sorolla, Ph.D. Thesis, Cornell University; *Dissertation Abstr.*, **20**, 1148 (1959); (c) A. Giner-Sorolla, *Galenica Acta* (Madrid), **19**, 97 (1966); *Chem. Abstr.*, **68**, 11303 (1968).

(3) J. J. Biesele, Proceedings of the 3rd National Cancer Conference, Detroit, Mich., 1956, p 405; *cf.* ref 2a.

(4) (a) A. C. Sartorelli and H. F. Upchurch, *Cancer Res.*, **23**, 1077 (1963); (b) A. C. Sartorelli, A. L. Bieber, P. K. Chang, and G. A. Fischer, *Biochem. Pharmacol.*, **13**, 567 (1964).

(5) P. F. Pecora and M. Earl Balis, *ibid.*, **13**, 1071 (1964).

(6) (a) A. Bendich, G. B. Brown, F. S. Philips, and J. B. Thiersch, *J. Biol. Chem.*, **183**, 267 (1950); (b) F. S. Philips, J. B. Thiersch, and A. Bendich, *J. Pharmacol. Exp. Ther.*, **104**, 20 (1952).

(7) (a) M. A. Stevens, A. Giner-Sorolla, H. W. Smith, and G. B. Brown, *J. Org. Chem.*, **27**, 567 (1962); (b) G. B. Brown, G. Levin, S. Murphy, A. Sele, H. C. Reilly, G. S. Tarnowski, F. A. Schmidt, and C. S. Stock, *J. Med. Chem.*, **8**, 190 (1965).

(8) G. B. Brown, *Progr. Nucleic Acid Res. Mol. Biol.*, **8**, 209 (1968).

(9) (a) Y. Shiasu and A. Ohta, *Gann*, **54**, 221 (1963); (b) C. E. Searle and D. L. Woodhouse, *Acta Unio Intern. Contra Cancrum*, **19**, 519 (1963); (c) H. Endo and F. Kume, *Gann*, **54**, 443 (1963); (d) Y. Shirasu, *Proc. Soc. Exptl. Biol. Med.*, **118**, 812 (1965); (e) M. Hozumi, S. Inuzuka, and T. Sugimura, *Cancer Res.*, **27**, 1378 (1967).

(10) K. Sugiura and G. B. Brown, in preparation.