Syntheses and Biological Evaluation of 2- and 9-Aminobenzonorbornenes as Conformationally Rigid Analogs of Amphetamines

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Abstract □ Isomers of the 2- and 9-aminobenzonorbornenes were prepared as rigid analogs of amphetamine and were employed to study the conformational requirements of indirectly acting sympathomimetic agents. Of this series of isomeric amines, the *exo*-2 and *anti*-9 isomers closely resemble the fully extended conformation of amphetamine. The other two amines, the *endo*-2 and *syn*-9 isomers, conformationally resemble the folded conformation of amphetamine. The isomers that resemble the extended conformation of amphetamine increased the spontaneous motor activity in mice while the isomers resembling the folded form either decreased or had no effect on motor activity. These compounds also were studied for their ability to accelerate the efflux of tritiated norepinephrine from vesicular and nonvesicular storage sites of isolated perfused rabbit atria; either α-methyl-*p*-tyrosine- or reserpine-pretreated rabbits were used. Amphetamine and the *exo*-2 and *anti*-9 isomers of aminobenzonorbornene could accelerate norepinephrine efflux from either compartment while the *endo*-2 and *syn*-9 isomers could accelerate the efflux from only the nonvesicular compartment at the concentrations studied. Fenfluramine and methylphenidate also were studied for their ability to accelerate efflux. Fenfluramine and methylphenidate resembled the aminobenzonorbornenes that correspond to the folded conformation of amphetamine in their ability to accelerate the efflux from nonvesicular storage. However, fenfluramine also resembled amphetamine and the aminobenzonorbornenes corresponding to the extended conformation of amphetamine in its ability to accelerate efflux from vesicular storage sites. The response to methylphenidate was similar to that of the aminobenzonorbornenes resembling the folded conformation of amphetamine.

Keyphrases □ Aminobenzonorbornenes—syntheses and biological evaluation as conformationally rigid analogs of amphetamines □ Amphetamines—syntheses and biological evaluation of the 2- and 9-aminobenzonorbornenes as conformationally rigid analogs □ Conformational requirements—indirectly acting sympathomimetic agents, syntheses and biological evaluation of the 2- and 9-aminobenzonorbornenes as amphetamine analogs

Amphetamine is a unique drug with respect to its simple structure and multiple pharmacological effects. Its wide spectrum of biological activity may be ascribed to its structural resemblance to several neurotransmitters such as norepinephrine, dopamine, and serotonin. One structural feature common to all of these compounds is an amino group separated by two carbon atoms from an aromatic ring. Free rotation about the two carbon atoms of such an ethylamine side chain permits amphetamine to assume suitable conformations to interact with various receptors in neurons associated with these neurotransmitters.

BACKGROUND

A conformational energy map of amphetamine, generated by Pullman *et al.*[1] (1), shows three energetically equivalent minima. One minimum corresponds to the fully extended form of amphetamine where the nitrogen atom is pointed away from the aromatic ring, and the other two minima correspond to folded forms of amphetamine where the nitrogen is rotated toward the aromatic ring (1).

Another major investigation concerning the conformation of phenethylamine derivatives was that by Weintraub and Hopfinger (2). They employed empirical potential functions, which take into account several aspects that are neglected in the quantum mechanical calculations such as solvent effects and the conformational dependence on the ionization state of the molecule. Their work is in agreement with that of Pullman *et al.* (1) in that the same two basic conformations, extended and folded, are predicted.

However, the work by Weintraub and Hopfinger suggests that the most probable conformation of amphetamine for a given ionization state of the amine nitrogen depends on the interaction with aqueous solvent. They calculated that the most stable conformation for unionized amphetamine in a vacuum is the folded form. However, for the charged molecule in aqueous media, their calculations predict a strong preference for the extended conformation (2). These findings of the conformational preference of amphetamine are in agreement with the results of Neville *et al.* (3), whose PMR study of amphetamine in aqueous solution showed the molecule to be in the extended form.

While such mathematical methods can provide a theoretical model for the preferred conformation of a molecule, certain aspects cannot be included in these calculations. One such aspect concerns the lack of knowledge concerning the precise nature of the drug–receptor interaction. Hence, the effects of the receptor on the drug conformation cannot be determined. The molecular level local environmental conditions also are unknown and, therefore, cannot be considered.

These limitations of theoretical methods for the determination of conformations clearly suggest a study of the biological activities of rigid analogs of amphetamine. The two major centers of the amphetamine molecule that are implicated most frequently in receptor interactions are the amino group and the aromatic ring. The distance between these two parts of the amphetamine molecule for each predicted stable conformation, as well as the corresponding distance for the 2- and 9-aminobenzonorbornenes, is shown in Table I.

The use of rigid analogs of a pharmacologically active compound can yield information about the stereochemical requirements for a molecule to elicit the biological response. In the case of amphetamine and its derivatives, the wide variety of physiological effects observed for these compounds may be due partially to the ability of the ethylamine chain to assume various conformations, permitting interaction with many types of receptors. To study the conformational requirements for the biological activity of amphetamine, the 2- and 9-aminobenzonorbornenes were designed and synthesized as conformationally rigid analogs of amphetamine. These compounds are strictly rigid, offer only a minimal addition to the molecular weight and size of the amphetamine molecule, and have

Table I—Atomic Parameters for Amphetamine and the Isomeric 2- and 9-Aminobenzonorbornenes

Compound	D_1[a], Å	D_2[b], Å
Amphetamine[c]		
Extended conformation	5.11	1.24
Folded conformation	3.82	2.14
2-Aminobenzonorbornenes[d]		
exo-Isomer, I	5.1	1.7
endo-Isomer, II	3.7	2.5
9-Aminobenzonorbornenes[d]		
anti-Isomer, III	4.8	0.7
syn-Isomer, IV	3.6	2.3

[a] Distance from the center of the aromatic ring to the amino nitrogen. [b] Distance from the plane of the aromatic ring to the amino nitrogen. [c] Distances as calculated by Pullman *et al.* (1). [d] Distances measured using Dreiding models.

[1] Using a refined all-valence-electrons procedure, designated as the PCILO method (perturbative configurational interaction using localized orbitals).

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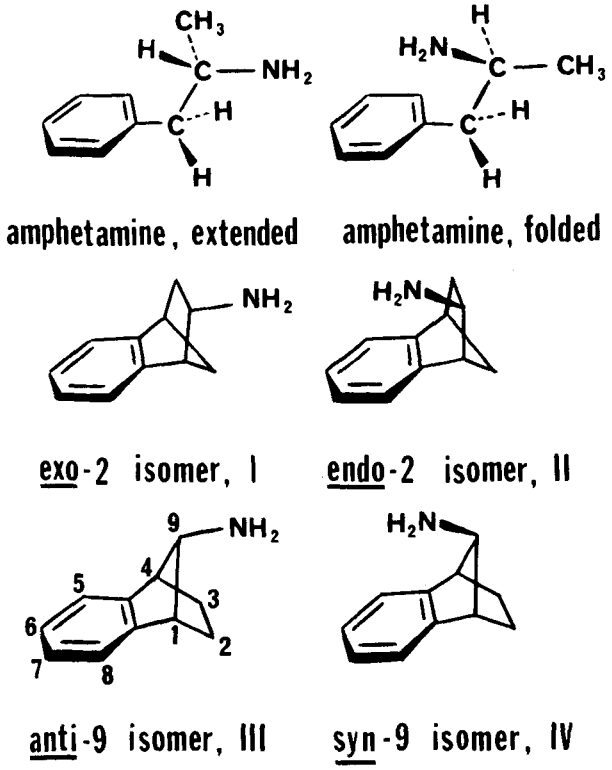

Figure 1—*Steric relationships between the 2- and 9-aminobenzonorbornenes and the stable conformers of amphetamine. The numbering of the benzonorbornene ring systems is shown on the* anti-*9 isomer.*

two isomers closely related conformationally to the folded form of amphetamine and two isomers closely related to the extended conformation of amphetamine. The relationship of these compounds to the predicted stable conformations of amphetamine is shown in Table I and Fig. 1. A preliminary account of this study was reported previously (4).

EXPERIMENTAL

Chemical[2]—exo-*2-Aminobenzonorbornene (I) Hydrochloride*—To a mixture of sodium borohydride (0.78 g, 0.02 mole) and benzonorbornadiene (5) (7.1 g, 0.05 mole) in diglyme (25 ml) at 0° (under nitrogen) was added dropwise, over 10 min, boron trifluoride etherate (3.90 g, 0.03 mole). The mixture then was stirred at 25° for 3 hr under nitrogen. A slurry of hydroxylamine *O*-sulfonic acid (10 g, 0.09 mole) in diglyme (45 ml) was added, and stirring was continued for 18 hr at 100°. The amber solution was decanted from insoluble material, treated with concentrated hydrochloric acid (20 ml) and water (200 ml), and extracted with ether (3 × 75 ml). The aqueous layer was made strongly alkaline with sodium hydroxide (15 g) and extracted with ether (3 × 75 ml).

The ether extract was washed with water (100 ml) and dried over anhydrous sodium sulfate. The solvents were removed *in vacuo*, first at 60° (50 torr) and then at 55° (0.5 torr), leaving *exo*-2-aminobenzonorbornene as a yellow oil (2.3 g, 30%); PMR: δ 1.15–2.08 (complex m, 4H, H-3 and H-9), 1.43 (s, 2H, exchangeable with deuterium oxide, NH_2), 2.80–3.10 (complex m, 2H, bridgehead protons), 3.17–3.36 (complex m, 1H, *endo*-H-2), and 7.04 (m, 4H, aromatic protons); mass spectrum: *m/z* (relative intensity) 159 (M^+, 17) and 116 (100). This amine was reportedly prepared by another method, but no experimental details or structure proof was given (6).

A solution of the amine (2.30 g) in anhydrous ether (50 ml) was treated with dry hydrogen chloride. The salt was collected and recrystallized quantitatively from absolute methanol–anhydrous ether (3:150 ml), mp 278–281° (sealed tube).

Anal.—Calc. for $C_{11}H_{14}ClN$: C, 67.52; H, 7.26; N, 7.16. Found: C, 67.69; H, 7.27; N, 7.12.

2-Benzonorbornenone Oxime—To a solution of hydroxylamine hydrochloride (1.86 g, 0.03 mole) and sodium acetate (2.79 g, 0.04 mole) in water (8 ml) were added 2-benzonorbornenone (7, 8) (3 g, 0.02 mole) and enough ethanol to effect dissolution (5 ml). This mixture was heated on a steam bath for 20 min. After cooling, the formed precipitate (2.4 g) was collected, and the mother liquor was extracted with chloroform (2 × 25 ml) to give an additional 0.6 g of the crude oxime. Recrystallization from ethanol (~7 ml) furnished the pure product (2.91 g, 89%), mp 118–119.5°; PMR: δ 1.95–2.95 (complex m, 4H, H-3 and H-9), 3.49–3.70 (m, 1H, H-4), 3.85–3.95 (m, 1H, H-1), 7.00–7.41 (m, 4H, aromatic protons), and 7.45–8.00 (m, 1H, exchangeable with deuterium oxide, N–OH); mass spectrum: *m/z* (relative intensity) 173 (M^+, 58), 116 (46), and 115 (100).

Anal.—Calc. for $C_{11}H_{11}NO$: C, 76.30; H, 6.43; N, 8.09. Found: C, 76.44; H, 6.40; N, 8.24.

endo-*2-Aminobenzonorbornene (II) Hydrochloride*—Sodium (2.5 g, 0.28 g atom) was added (in four pieces) to a stirred solution of the oxime (1.73 g, 0.01 mole) in 1-propanol (250 ml) over 45 min. After the sodium was consumed, the solvent was removed *in vacuo*. The thick residue was diluted with water (500 ml) and extracted with ether (3 × 100 ml). The ether extract was dried over anhydrous sodium sulfate and evaporated *in vacuo* to give the amine (1.42 g, 90%) as an oil; PMR: δ 0.37–0.68 (m, 1H, *endo*-H-3), 0.98 (s, 2H, exchangeable with deuterium oxide, NH_2), 1.61–1.92 (complex m, 2H, H-9), 2.05–2.56 (complex m, 1H, *exo*-H-3), 3.03–3.28 (complex m, 2H, bridgehead protons), 3.43–3.78 (complex m, 1H, *exo*-H-2), and 6.95–7.33 (m, 4H, aromatic protons); mass spectrum: *m/z* (relative intensity) 159 (M^+, 17) and 116 (100). This amine has been reported, but no experimental details or structure proof was provided (9).

The amine was converted to its hydrochloride as already described, mp 220–223°.

Anal.—Calc. for $C_{11}H_{14}ClN$: C, 67.52; H, 7.26; N, 7.16. Found: C, 67.78; H, 7.26; N, 7.28.

9-Benzonorbornenone Oxime—To a solution of hydroxylamine hydrochloride (2.82 g, 0.04 mole) and sodium acetate (4.20 g, 0.05 mole) in water (12 ml) were added 9-benzonorbornenone (10, 11) (4.50 g, 0.02 mole) and enough ethanol to effect dissolution (~10 ml). This mixture was heated on a steam bath for 4 hr, cooled, and extracted with ether (3 × 25 ml). The ether extract was washed with water, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to give 9-benzonorbornenone oxime as a thick amber oil. This oil could not be crystallized readily and thus was used in subsequent reactions without further purification.

syn-*9-Aminobenzonorbornene (IV) and* anti-*9-Aminobenzonorbornene (III) Hydrochlorides*—Sodium (16 g, 0.7 g atom) was added in three pieces to a solution of 9-benzonorbornenone oxime (4.00 g, 0.023 mole) in 1-propanol (600 ml) over 1.5 hr. After the sodium was consumed completely, the mixture was cooled and acidified with concentrated hydrochloric acid, and the solvents were removed *in vacuo*. The residue was diluted with water (200 ml) and extracted with benzene to remove neutral and acidic substances. The aqueous layer was made basic with sodium hydroxide and extracted with benzene.

The benzene extract was dried over anhydrous magnesium sulfate and evaporated *in vacuo* to give a mixture of *syn*-9 and *anti*-9-amines (3.16 g, 86%). This mixture was dissolved in formic acid (150 ml) and treated with acetic anhydride (150 ml) over 10 min, followed by a 3-hr reflux to prepare the corresponding formamides. Removal of the solvents *in vacuo* gave the formamides (3.47 g, 93%); PMR: δ 7.86 and 8.10 (s, ~0.5H each, CHO).

This formamide mixture was chromatographed on a column of silica gel[3] (600 g) prepared in benzene. The *syn*-9-amide (1.39 g) was eluted with benzene–ether (2:1, ~1.5 liters) and recrystallized from 2-propanol, mp 129–131°; PMR: δ 1.01–1.33 (m, 2H, *endo*-H-2 and *endo*-H-3), 1.92–2.29 (m, 2H, *exo*-H-2 and *exo*-H-3), 3.27 (m, 2H, bridgehead protons), 4.14–4.29 (m, 1H, *anti*-H-9), 5.19–5.95 (broad s, 1H, NH), 7.17 (m, 4H, aromatic protons), and 7.86 (s, 1H, CHO); mass spectrum: *m/z* (relative intensity) 188 ($M^+ + 1$, 3), 187 (M^+, 9), and 142 (100).

Anal.—Calc. for $C_{12}H_{13}NO$: C, 76.97; H, 7.00; N, 7.48. Found: C, 77.15; H, 7.02; N, 7.39.

The *anti*-9-amide (1.77 g) also was eluted with benzene–ether (2:1, ~2

[2] Melting points below 240° were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected, and those over 240° were determined on a Mel-Temp apparatus and are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Ill. PMR spectra were obtained in deuterochloroform on a Varian A-60 spectrometer equipped with a Nicolet TT-7 Fourier transform accessory. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as the internal standard. Mass spectra (at 70 ev) were obtained by Richard Dvorak using a Hitachi Perkin-Elmer RMU-D6 single-focusing mass spectrometer.

[3] J. T. Baker Chemical Co., 60–200 mesh.

liters) and recrystallized from ethanol, mp 133.5–135°; PMR: δ 1.11–1.36 (m, 2H, *endo*-H-2 and *endo*-H-3), 1.86–2.17 (m, 2H, *exo*-H-2 and *exo*-H-3), 3.28 (m, 2H, bridgehead protons), 3.85–3.96 (m, 1H, *syn*-H-9), 6.17–6.70 (broad s, 1H, NH), 7.14 (m, 4H, aromatic protons), and 8.10 (s, 1H, CHO); mass spectrum: *m/z* (relative intensity) 188 ($M^+ + 1$, 3), 187 (M^+, 9), and 142 (100).

Anal.—Calc. for $C_{12}H_{13}NO$: C, 76.97; H, 7.00; N, 7.48. Found: C, 77.19; H, 7.08; N, 7.45.

A solution of the *syn*-9-formamide (1.0 g) in 10% HCl (30 ml) was heated under reflux for 1 hr. The aqueous solution was extracted with ether (3 × 25 ml), made basic with sodium hydroxide pellets (with cooling), and extracted with benzene (3 × 50 ml). The benzene extract was dried over anhydrous magnesium sulfate and evaporated *in vacuo* to give *syn*-9-aminobenzonorbornene quantitatively; PMR: δ 0.87–1.33 (complex m, 2H, *endo*-H-2 and *endo*-H-3), 0.98 (s, 2H, exchangeable with deuterium oxide, NH_2), 1.80–2.18 (complex m, 2H, *exo*-H-2 and *exo*-H-3), 2.97–3.13 (complex m, 2H, bridgehead protons), 3.29 (s, 1H, *anti*-H-9), and 7.06–7.30 (m, 4H, aromatic protons); mass spectrum: *m/z* (relative intensity) 159 (M^+, 18) and 128 (100).

The amine was converted to its hydrochloride as already described, mp 344–346° (sealed tube).

Anal.—Calc. for $C_{11}H_{14}ClN$: C, 67.52; H, 7.26; N, 7.16. Found: C, 67.79; H, 7.26; N, 7.05.

The *anti*-9-formamide (1.0 g) was hydrolyzed as already described to yield *anti*-9-aminobenzonorbornene quantitatively; PMR: δ 1.02–1.40 (complex m, 2H, *endo*-H-2 and *endo*-H-3), 1.24 (s, 2H, exchangeable with deuterium oxide, NH_2), 1.87–2.27 (complex m, 2H, *exo*-H-2 and *exo*-H-3), 2.97–3.20 (m, 3H, *syn*-H-9 and bridgehead protons), and 7.14 (m, 4H, aromatic protons), in good agreement with the literature data (12, 13); mass spectrum: *m/z* (relative intensity) 159 (M^+, 35) and 128 (100).

The hydrochloride was prepared as already described, mp 298–302° (sealed tube) [lit. (12) mp > 240°].

Anal.—Calc. for $C_{11}H_{14}ClN$: C, 67.52; H, 7.26; N, 7.16. Found: C, 67.60; H, 7.22; N, 7.11.

Biological[4]—*Evaluation of Effect on Motor Activity*—The effect of the 2- and 9-aminobenzonorbornenes on spontaneous motor activity was determined using groups of five female ICR mice (17–22 g). Three circular activity cages, each containing six photocells, were used. To eliminate error due to any difference in sensitivity of the photocells, each dose of each compound was tested once in each cage and the values were averaged. All drugs and controls were administered intraperitoneally in a dose volume of 1–1.5 ml/100 g of weight. The aminobenzonorbornene hydrochlorides were administered as isotonic saline solutions.

Control animals were treated with appropriate volumes of isotonic saline. Controls were carried out each testing day in each cage and averaged. The cages were dark inside, and ambient noise levels were kept to a minimum. Counting began immediately after injection and was continued for 30 min.

Evaluation of Ability to Accelerate Efflux of Tritiated Norepinephrine from Isolated Perfused Rabbit Atria—The experiment was designed to measure the ability of the aminobenzonorbornenes (and several known sympathomimetics) to accelerate the efflux of tritium-labeled norepinephrine from isolated perfused rabbit atria. The technique represents a modification of that reported by Paton (14).

Adult male New Zealand rabbits, 1.8–3.0 kg, were pretreated with either reserpine (2.0 mg/kg sc, 18 hr; 1.0 mg/kg iv, 1 hr) or D,L-α-methyl-*p*-tyrosine (250 mg/kg ip, 9 hr). The animals were killed by a blow on the neck, and their hearts then were excised rapidly. The atria were isolated and cut into six pieces of ~35 mg each. The pieces then were mounted on fine hooks (prepared by bending the needles of standard 1-ml tuberculin syringes), which held the tissues and also delivered a constant stream of oxygen to any medium into which the tissues were placed.

The atrial dissection was performed under constantly oxygenated medium (100% oxygen) at 25°, and all subsequent incubations were done at 37°. The medium had the following composition: 140 m*M* NaCl, 5 m*M* KCl, 1.5 m*M* $CaCl{\cdot}H_2O$, 1.2 m*M* $MgSO_4$, 10 m*M* tris(hydroxymethyl)-aminomethane (pH 7.4), and 10 m*M* D-glucose. Edetate disodium dihydrate (ethylenediaminetetraacetic acid disodium salt dihydrate) (3×10^{-5} *M*) and sodium ascorbate (1×10^{-4} *M*) were added to the medium to prevent norepinephrine oxidation. To inhibit catechol *O*-methyltransferase, the medium contained 1×10^{-4} *M* tropolone (14).

Once the atrial pieces were mounted onto the hooks, they were allowed to stand in the medium for 30 min to equilibrate. Then the tissue pieces were incubated for 30 min in medium that contained pargyline (5×10^{-4} *M*) to inhibit monoamine oxidase. The tissues then were transferred to fresh medium for 30 min to wash out the excess pargyline and were incubated in medium containing 5.8×10^{-7} *M* tritiated norepinephrine for 1 hr. The tissues then were transferred every 5 min successively through a series of 21 tubes, each containing 5 ml of fresh medium. The compounds studied were present from 60 to 100 min during which efflux was measured. Amphetamine, methylphenidate, isomer I, and isomer III were studied at 5×10^{-5} *M*; fenfluramine, isomer II, and isomer IV were studied at 1×10^{-4} *M*. At the end of the efflux period, the tissues were placed into scintillation vials containing 1 ml of tissue solubilizer at 37°. Once the tissues were solubilized, the tissue solubilizer was neutralized with acetic acid, 10 ml of liquid scintillation cocktail was added, and the total tritium content was determined.

One-milliliter portions of all efflux media (tubes 1–21) were added to scintillation vials, 10 ml of scintillation cocktail was added, and the tritium content was determined. In all cases, the scintillation counting was done after the vials had been cooled and dark adapted. The average counting efficiency was 40%.

The tritiated norepinephrine content (A_t) of the tissue at various times was calculated by adding successively, in reverse order, the amount of tritiated norepinephrine released into each tube during efflux to that remaining in the tissue at the end of the experiment. Efflux was expressed as a rate coefficient (f) in minutes^{-1}:

$$f = \frac{\Delta A}{\Delta t A_t} \qquad \text{(Eq. 1)}$$

where ΔA represents the decays per minute (dpm) lost in the time interval Δt (5 min) and A_t is the amount of tritiated norepinephrine (dpm) in the tissue at the midpoint of the time interval Δt.

RESULTS

Effect of 2- and 9-Aminobenzonorbornenes on Spontaneous Motor Activity—The effects of the aminobenzonorbornenes on spontaneous motor activity are shown in Fig. 2. Isomers I and III increased motor activity while II decreased motor activity. The *syn*-9 isomer (IV) did not significantly affect motor activity. The effect of dextroamphetamine sulfate also was studied by the same procedure for direct comparison to the aminobenzonorbornenes. The ED_{50} of dextroamphetamine sulfate was 0.55 mg/kg, and the maximal response was similar to that obtained for *anti*-9-aminobenzonorbornene.

Acceleration of Efflux of Tritiated Norepinephrine from Isolated Perfused Rabbit Atria—The results are shown in Figs. 3 and 4 in which the calculated rate coefficient is plotted against time. In Fig. 3A, the entire efflux period is plotted. In all subsequent figures, only the efflux from 35 to 105 min after labeling is shown. In all cases, the portion of the graph not included is similar to that of Fig. 3A.

All four 2- and 9-aminobenzonorbornene isomers accelerated the efflux of tritiated norepinephrine from neurons of reserpine-treated animals. Isomers I and III exhibited effects similar in magnitude and duration to those caused by amphetamine. There was a peak response in the first tube (55–60 min), which contained the most tritiated norepinephrine after efflux, after which the amount of tritiated norepinephrine released into each tube approached control levels.

Isomers II and IV were comparatively much less potent at the concentration studied. These isomers were also similar to each other in magnitude and duration of response. They were, however, considerably different from I and III in the time course of response. Whereas I and III caused an immediate maximal response which rapidly fell back toward control levels, it took several tubes for II and IV (55–70 min) before the maximal response was noted; and once this maximum level was reached, it was maintained for the duration of efflux. This finding was similar to the results obtained for fenfluramine and methylphenidate.

When the animals were pretreated with α-methyl-*p*-tyrosine, I and III, as well as amphetamine, again produced an immediate maximal response. The magnitude and time course of the response caused by I and III were similar to these caused by amphetamine. In contrast to reserpine pretreatment, the response did not fall rapidly after the immediate maximum but declined only slightly from the maximal response during the remainder of efflux.

Isomers II and IV were much less potent at accelerating the efflux of

[4] Injectable reserpine (Serpasil) was purchased from Ciba Pharmaceutical Co. Pargyline hydrochloride and D,L-α-methyl-*p*-tyrosine were purchased from Sigma Chemical Co. The α-methyl-*p*-tyrosine was administered as a suspension in corn oil. NCS tissue solubilizer was purchased from Amersham/Searle. Tritiated L-norepinephrine (5.8 Ci/mmole, NET-377) was purchased from New England Nuclear. All scintillation counting was done in 22-mm glass vials (Research Products International Corp., Elk Grove Village, Ill., No. 121053, polyethylene-lined caps) with a Packard model 2425 Tri-Carb liquid scintillation spectrometer. The scintillation cocktail was Packard Insta-Gel (6002177).

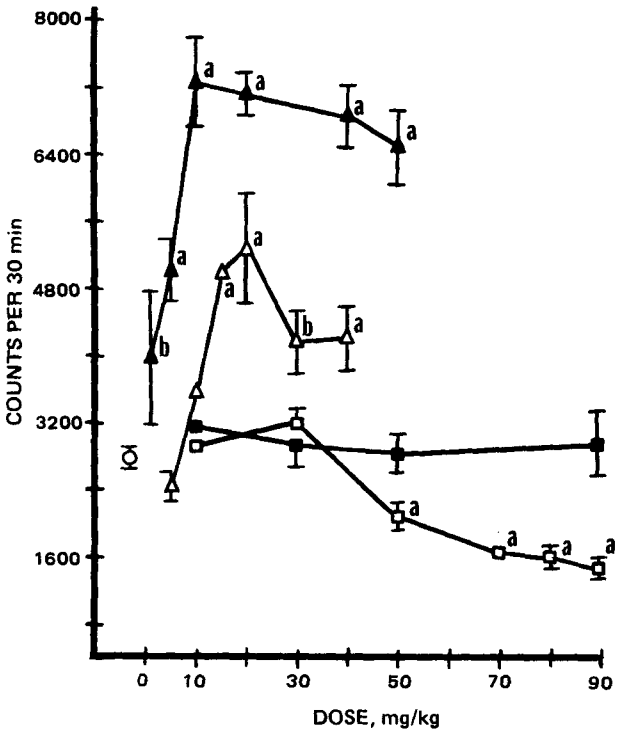

Figure 2—*Effect of the isomeric aminobenzonorbornenes on the spontaneous motor activity of mice. The data are expressed as counts per 30 min* versus *various doses of the compounds; the procedures are described under* Experimental. *Each point represents the mean of three experiments of five mice each (±SEM), except the control value (○) where* n = *33. Key: △, isomer I; □, isomer II; ▲, isomer III; and ■, isomer IV.*

tritiated norepinephine from atria of α-methyl-*p*-tyrosine-pretreated rabbits. They did produce a very slight immediate increase in efflux, which then decreased to nearly control levels. Fenfluramine was approximately as effective at accelerating the efflux of tritiated norepinephrine from α-methyl-*p*-tyrosine-pretreated tissues as from reserpine-pretreated tissue. Methylphenidate resembled II and IV in that it was capable of accelerating the efflux of tritiated norepinephrine from reserpine-pretreated tissues but not from α-methyl-*p*-tyrosine-pretreated tissues.

DISCUSSION

Of the aminobenzonorbornenes studied, only isomers that resemble the extended conformation of amphetamine (I and III) increased the spontaneous motor activity of mice. These results are consistent with an earlier study which led to the conclusion that the extended conformation is critical for an agent of this type to cause an increase in motor activity (15). That isomer II decreased motor activity while IV had no effect on it is in general agreement with previous studies in which rigid and semirigid analogs that resembled the folded conformation of amphetamine either decreased or had no effect on motor activity (15, 16).

It is generally accepted that functionally distinct compartmentation of stored norepinephrine exists in adrenergic neurons. This compartmentation is believed to consist primarily of a reserpine-sensitive vesicular store and a reserpine-resistant extravesicular (cytoplasmic) store (17–21). The cytoplasmic level of accumulated norepinephrine normally is regulated by vesicular storage (17) and by deamination by monoamine oxidase (22–24). Therefore, inhibition of these processes provides a means of increasing the size of the cytoplasmic store. In the present study, the ability of the isomers of aminobenzonorbornene to accelerate the efflux of tritiated norepinephrine from these two major compartments was studied. By using pargyline-treated atrial tissue from reserpine-pretreated rabbits, a primarily cytoplasmic store was obtained. A primarily vesicular storage was obtained by using atrial tissue from α-methyl-*p*-tyrosine-pretreated rabbits. Although pargyline also was used in the α-methyl-*p*-tyrosine cases, the evidence indicates the lack of a significant cytoplasmic store.

In another recent study using rigid analogs of amphetamine and a

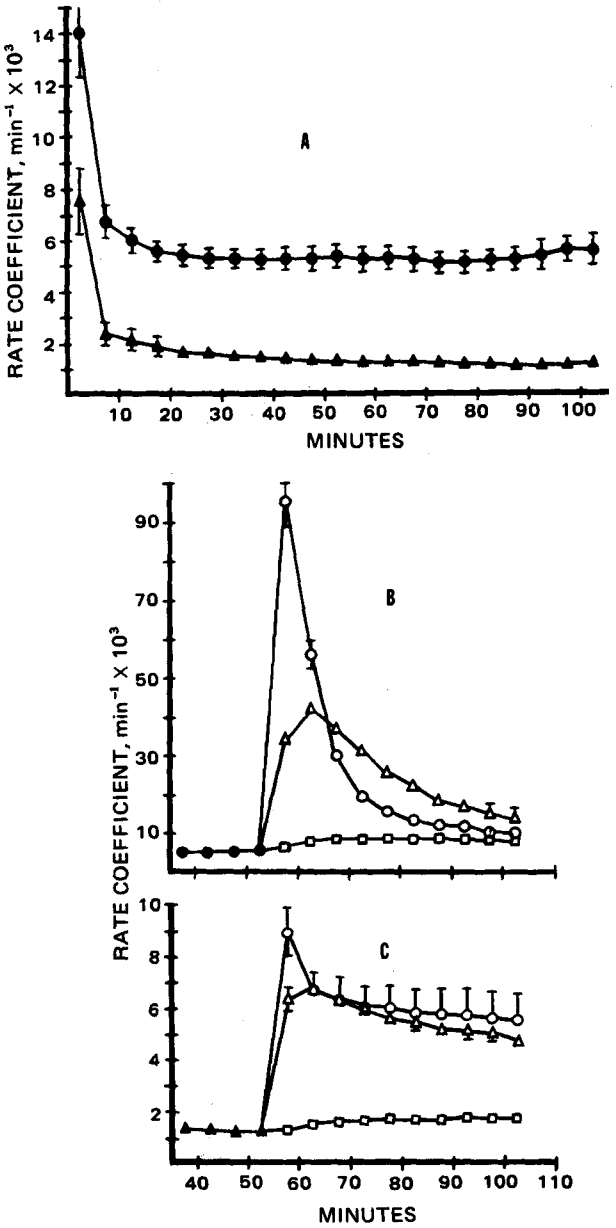

Figure 3—*Efflux of tritiated norepinephrine from isolated perfused rabbit atria; the procedures are described under* Experimental. *Key for graph A, controls* (n = *5):* ●, *reserpine pretreatment; and* ▲, *α-methyl-*p*-tyrosine pretreatment. Key for graph B, reserpine pretreatment* (n = *3), and graph C, α-methyl-*p*-tyrosine pretreatment* (n = *3):* ○, *amphetamine, 5* × 10^{-5} M; △, *fenfluramine, 1* × 10^{-4} M; *and* □, *methylphenidate, 5* × 10^{-5} M.

similar experimental protocol, it was concluded that the fully extended form of amphetamine is involved in the displacement of norepinephrine from the cytoplasmic compartment while conformational mobility, or a conformation not approximated by either reported isomer, is required for displacement from the vesicular compartment.(25).

In the present study, all of the aminobenzonorbornenes were capable of accelerating the efflux of tritiated norepinephrine from reserpine-pretreated tissue. This finding indicated that each amine was capable of entering the neurons. The difference in the manner in which these rigid analogs accelerate the efflux of tritiated norepinephrine may reflect different types of tritiated norepinephrine storage with different stereochemical sensitivities and/or different modes of entry (for the test compounds) into the neurons (active transport *versus* passive diffusion). The curve shape and the magnitude of maximal response for methylphenidate were similar to those of II and IV. This fact may indicate that the stable conformation of methylphenidate resembles the folded form of amphetamine.

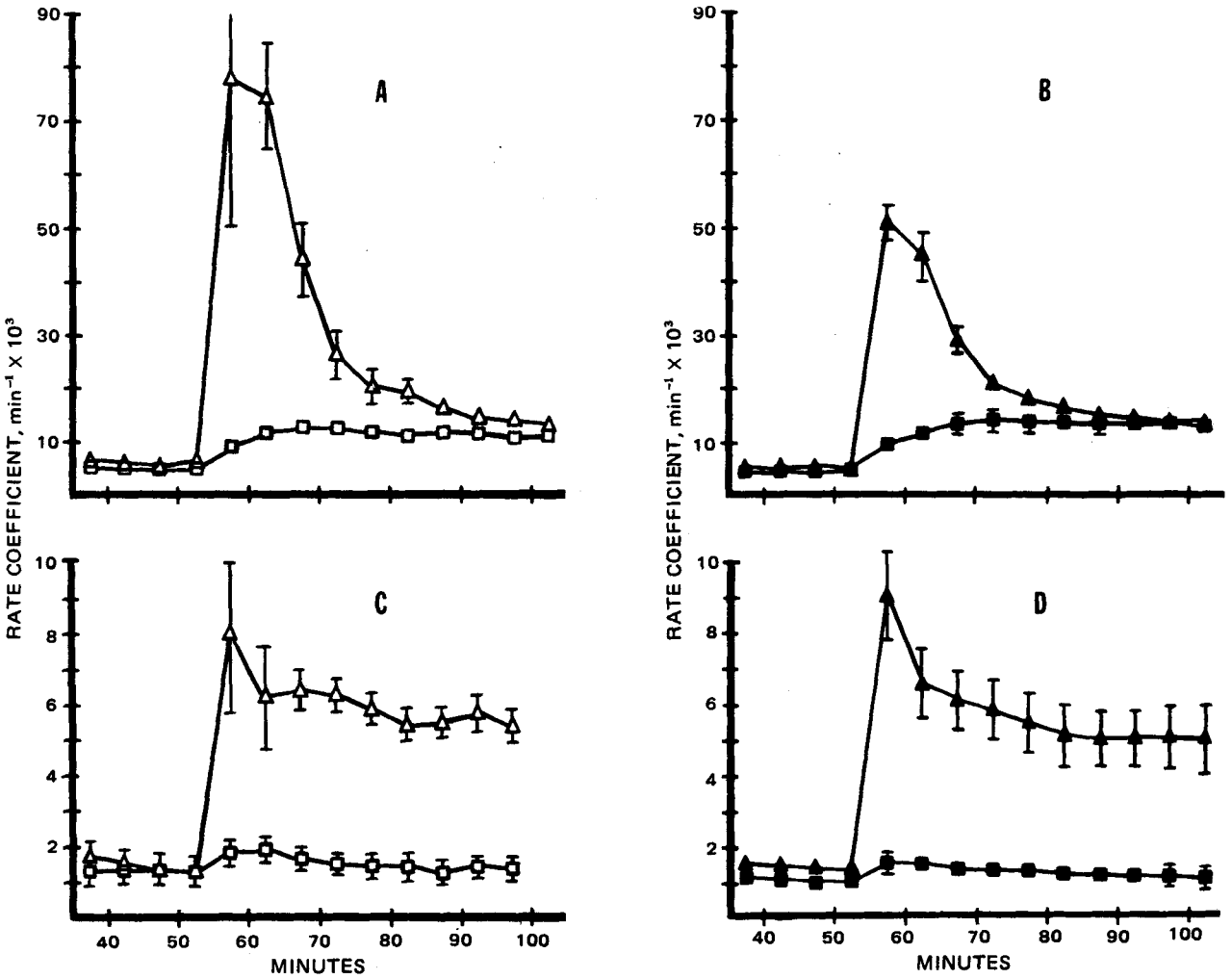

Figure 4—*Efflux of tritiated norepinephrine from isolated perfused rabbit atria (n = 3); the procedures are described under* Experimental. *Graphs A and B are for reserpine pretreatment, and graphs C and D are for α-methyl-*p*-tyrosine pretreatment. Key: △, isomer I, 5 × 10⁻⁵ M; □, isomer II, 1 × 10⁻⁴ M; ▲, isomer III, 5 × 10⁻⁵ M; and ■, isomer IV, 1 × 10⁻⁴ M.*

When the tissue used for these studies was taken from animals pretreated with α-methyl-*p*-tyrosine, the results were quite different. Again, rigid analogs resembling the extended form of amphetamine were able to release tritiated norepinephrine. Also the shape of the curve and the magnitude of the response were similar for each compound (I and III and amphetamine). The analogs resembling the folded conformation, however, were unable to release tritiated norepinephrine significantly from the tissue of α-methyl-*p*-tyrosine-treated animals. The use of the two pretreatments (reserpine *versus* α-methyl-*p*-tyrosine) to give either predominantly cytoplasmic or vesicular storage of the perfused tritiated norepinephrine indicates that release from the vesicular storage has more rigid stereochemical requirements than release from cytoplasmic (reserpine insensitive) sites. The extended conformation of amphetamine appears to be necessary for release from the vesicular sites while either the extended or the folded forms may cause the release from cytoplasmic sites. That methylphenidate again resembles II and IV, this time in its inability to accelerate efflux from α-methyl-*p*-tyrosine-pretreated tissues, further indicates that the stable conformation of methylphenidate may resemble the folded form of amphetamine.

It was reported that fenfluramine causes a decrease in motor activity except in animals pretreated with a monoamine oxidase inhibitor, in which case fenfluramine is nearly equipotent with amphetamine in increasing motor activity (26). In comparing the efflux data for these two compounds, one possible explanation for this phenomenon may be seen. Both compounds have similar curves for releasing tritiated norepinephrine from α-methyl-*p*-tyrosine-pretreated animals with the exception that amphetamine shows a slight maximal response, which then falls rapidly to a constant level of efflux, while fenfluramine comes to this level immediately and declines only slightly from this level. However, in reserpine-pretreated tissue, amphetamine shows a much stronger initial response than does fenfluramine. This ability of fenfluramine to release tritiated norepinephrine from vesicles as effectively as amphetamine, while not being as effective at releasing tritiated norepinephrine from cytoplasmic sites, would cause the norepinephrine released from vesicles, by fenfluramine, to have a greater exposure to intraneuronal monoamine oxidase than that released by amphetamine. If the level of norepinephrine in the cytoplasm and the level of deaminated metabolites of norepinephrine inside the neuron have a biochemical role in the neuron, it can be seen how compounds that can displace norepinephrine from cytoplasmic sites but not from vesicular sites (and vice versa) may derive some pharmacological effects *via* alteration of the normal level of these metabolites and nonvesicular norepinephrine.

REFERENCES

(1) B. Pullman, J.-L. Coubeils, P. Courrière, and J.-P. Gervois, *J. Med. Chem.*, **15**, 17 (1972).

(2) H. J. R. Weintraub and A. J. Hopfinger, *J. Theor. Biol.*, **41**, 53 (1973).

(3) G. A. Neville, R. Deslauriers, B. J. Blackburn, and I. C. Smith, *J. Med. Chem.*, **14**, 717 (1971).

(4) L. E. Wood, *Res. Commun. Chem. Pathol. Pharmacol.*, **21**, 169 (1978).

(5) G. Wittig and E. Knauss, *Chem. Ber.*, **91**, 895 (1958).

(6) P. Burn, P. A. Crooks, and J. M. H. Rees, *J. Pharm. Pharmacol., 12-Suppl.*, **28**, 80P (1976).

(7) P. D. Bartlett and W. P. Giddings, *J. Am. Chem. Soc.*, **82**, 1240 (1960).

(8) D. J. Sandman, K. Mislow, W. P. Giddings, J. Dirlam, and G. C. Hanson, *ibid.*, **90**, 4877 (1968).

(9) K. R. Bharucha, D. Ajdukovic, V. Pavilanis, and A. C. MacKay, U.S. pat. 3,932,512 (1976); through *Chem. Abstr.*, **84**, 135372c.
(10) P. F. Ranken and M. A. Battiste, *J. Org. Chem.*, **36**, 1996 (1971).
(11) R. Muneyuki and H. Tanida, *ibid.*, **31**, 1988 (1966).
(12) H. Tanida, T. Tsuji, and T. Irie, *ibid.*, **31**, 3941 (1966).
(13) J. Ehrenfreund and E. Zbiral, *Justus Liebigs Ann. Chem.*, **1973**, 290.
(14) D. M. Paton, *Br. J. Pharmacol.*, **49**, 614 (1973).
(15) E. E. Smissman and T. L. Pazdernik, *J. Med. Chem.*, **16**, 14 (1973).
(16) *Ibid.*, **16**, 18 (1973).
(17) N. Weiner, P. R. Draskoczy, and W. R. Burack, *J. Pharmacol. Exp. Ther.*, **137**, 47 (1962).
(18) M. J. Antonaccio and C. B. Smith, *ibid.*, **188**, 654 (1974).
(19) J. R. Crout, A. J. Muskus, and U. Trendelenburg, *Br. J. Pharmacol.*, **18**, 600 (1962).
(20) L.-O. Farnebo, *Biochem. Pharmacol.*, **20**, 2715 (1971).
(21) R. F. Furchgott, S. M. Kirpekar, M. Rieder, and A. Schwab, *J. Pharmacol. Exp. Ther.*, **142**, 39 (1963).
(22) A. Giachetti and R. A. Hollenbeck, *Br. J. Pharmacol.*, **58**, 497 (1976).
(23) A. Giachetti and P. R. Shore, *Biochem. Pharmacol.*, **15**, 607 (1966).
(24) L. L. Iversen, J. Glowinski, and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **150**, 173 (1965).
(25) J. A. Ruth, G. L. Grunewald, and C. O. Rutledge, *ibid.*, **204**, 615 (1978).
(26) R. J. Ziance, I. G. Sipes, W. J. Kinnard, Jr., and J. P. Buckley, *ibid.*, **180**, 110 (1972).

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Theophylline Magnesium Salicylate, a New Xanthine Compound

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Received February 19, 1980, from the *Pharmaceutical R&D Department, Hoffmann-La Roche Inc., Nutley, NJ 07110.* Accepted for publication August 1, 1980. *Present address: Block Drug Co., Jersey City, NJ 07302.

Abstract □ Interaction of 1 mole of magnesium salicylate and 2 moles of theophylline in water precipitated a crystalline compound, identified as theophylline magnesium salicylate pentahydrate from analytical and supportive physicochemical data. Similarly, barium salicylate and theophylline produced theophylline barium salicylate. No precipitates were formed with calcium salicylate or strontium salicylate under the same conditions. Theophylline magnesium salicylate is not a mixture of components and differs in composition from the known theophylline calcium salicylate dihydrate. Unlike the latter compound, it is not alkaline.

Keyphrases □ Theophylline magnesium salicylate—synthesis and properties □ Xanthines—theophylline magnesium salicylate, synthesis and properties

The methylxanthines theobromine, theophylline, and caffeine have been used to stimulate the central nervous system, to promote diuresis, and for their cardiovascular effects. While investigating theophylline as a diuretic component for a proprietary product, an insoluble product resulted from the interaction of magnesium salicylate and theophylline. This paper describes the preparation and properties of theophylline magnesium salicylate, a crystalline compound not previously reported.

BACKGROUND

Theobromine, theophylline, and caffeine are used medically as single entities, as soluble amine salts, or as the so-called double salts with alkali or alkaline earth metal salts of organic acids such as acetic, gluconic, benzoic, and salicylic acids. These double salts, believed by some to be definite compounds and by others to be simply mixtures, were prepared either to solubilize the xanthine (*e.g.*, theophylline sodium acetate and caffeine sodium benzoate) or to make insoluble products (*e.g.*, theobromine and theophylline calcium salicylates); the latter type of compound is claimed to be tolerated better in the GI tract than the parent xanthine.

Various methods have been described for the preparation of the xanthine double salts with alkali or alkaline earth metal salts of organic acids. For example, the soluble compound theophylline sodium acetate is made by heating together 1 equivalent each of theophylline and sodium hydroxide, mixing them with 1 equivalent of aqueous sodium acetate, and evaporating the mixture to dryness (1). Theobromine sodium salicylate is made in a similar fashion (2). Theobromine and theophylline calcium salicylates are prepared according to a 1925 patent (3) by dissolving the xanthine in aqueous sodium hydroxide, adding a solution of sodium salicylate followed by solutions of calcium chloride and ammonium hydroxide, and removing and drying the precipitate. The two insoluble calcium salicylate double salts are described as having the following compositions:

1. For the neutral double salt, $(C_7H_7N_4O_2)Ca(C_6H_4OHCO_2)_2Ca$. The present authors believe this empirical formula to be in error because the formula should be $(C_7H_7N_4O_2)_2Ca(C_6H_4OHCO_2)_2Ca$ or simply $(C_7H_7N_4O_2)Ca(C_6H_4OHCO_2)$ since a subsequent patent (4) indicated that the compound contains one atom of calcium and 1 mole each of theobromine and salicylic acid.
2. For the basic double salt, $(C_7H_7N_4O_2)_2Ca(C_6H_4OHO_2Ca)_2$.

The aforementioned patents also stated that the analogous strontium double salts can be made in a similar fashion.

Another patent (5), issued in 1949 for the same manufacturer as the previously cited patents, contained only one example for the preparation of neutral theobromine calcium salicylate[1] (by reacting theobromine calcium and salicylic acid), which is assigned the formula $(C_7H_7N_4O_2)$-$Ca(C_6H_4OHCO_2)\cdot H_2O$. The corresponding theophylline calcium salicylate[2] is the double salt of calcium salicylate and calcium theophylline in equimolecular proportions or $(C_7H_7N_4O_2)Ca(C_6H_4OHCO_2)\cdot 2H_2O$ (6). Both theobromine and theophylline calcium salicylates are slightly soluble in water and are alkaline (7–9).

This description of the formula and preparation of theophylline cal-

[1] Theocalcin, Knoll.
[2] Phyllicin, Knoll.

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