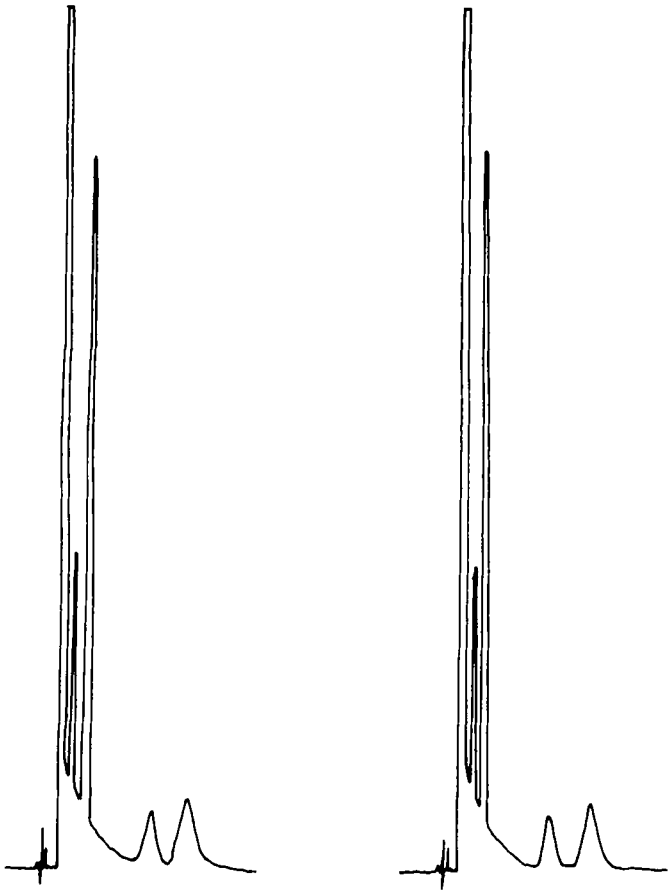

**Figure 5** —*Duplication of the chromatographic column.*

low-density polyethylene. The average thickness for the materials were 1.26 mm for polystyrene and high-density polyethylene and 1.00 mm for low-density polyethylene.

When using polystyrene for manufacturing plastic components which are to be sterilized with ethylene oxide, there is the possibility of a "mesh" effect due to the composite nature of the plastic; the presence of bulky phenyl groups gives the plastic many interstitial spaces. Ethylene oxide may enter these spaces within the plastic and remain there, dissipating slowly over a long time period. Obviously, elevated temperature and reduced pressure will increase the rate of dissipation. Likewise, high-density polyethylene plastic components retain more ethylene oxide than low-density polyethylene plastics due to their higher density, where it is less likely that the fumigant will penetrate the plastic.

A most satisfying feature of this method has been the extraordinary longevity of the column. This method of analysis has been used routinely for 18 months with only two replacement columns, giving ~1000 injections per column life. The end of the useful life of a column manifests itself in the broadening of the ethylene glycol peak and a drift in retention times. Duplication of the chromatographic column has been achieved several times with no adverse effects on the separation of the components (Fig. 5). Since this method uses only one isothermal column run for the analysis of all three components and no elaborate sample preparation is necessary, it would appear that this method is ideal for use in quality control laboratories of companies which need to comply with the Food and Drug Administration proposals.

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# Anti-Influenza A Activity of Some *N*-Substituted Bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine Hydrochlorides: Synthesis and Structure


**ELENA DE LA CUESTA, PALOMA BALLESTEROS, and GREGORIO G. TRIGO**[x]



Received March 9, 1983, from the *Departamento de Quimica Organica y Farmaceutica, Facultad de Farmacia, Universidad Complutense, Madrid-3, Spain.* Accepted for publication July 19, 1983.



**Abstract** □ Some *N*-substituted bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine hydrochlorides (IX–XII) prepared from bicyclo[3.2.1]octan-3-one (I), were assayed *in vitro* against influenza A viruses. All materials showed activity similar to 1-adamantanamine hydrochloride. A $^1$H-NMR study revealed only one isomer at the spiro carbon atom.



**Keyphrases** □ *N*-Substituted bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine hydrochlorides — synthesis, structure, antiviral activity against influenza A □ Antiviral agents—potential, *N*-substituted bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine hydrochlorides, influenza A


Since Davis *et al.* (1) described the antiviral activity of 1-adamantanamine (amantadine) against influenza A viruses, a wide variety of derivatives have been prepared (for review; 2). Among them, some spiropyrrolidine derivatives of adamantane and other cyclic systems (3, 4) have been reported to show amantadine-like antiviral activity.

In this report we describe the synthesis of the *N*-substituted bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine hydrochlorides


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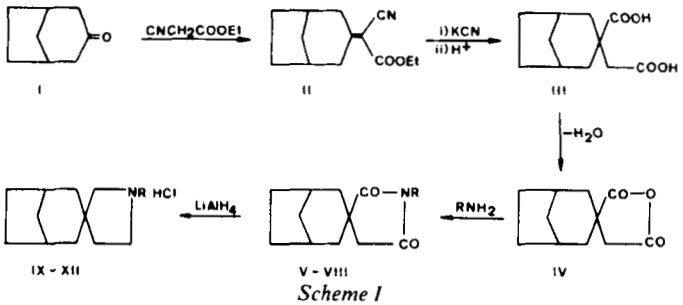


*Scheme I*

(IX-XII) (Scheme I) and their evaluation as potential antiviral agents.

## EXPERIMENTAL SECTION[1]

**Chemistry**—Bicyclo[3.2.1]octan-3-one (I) was obtained according to a general literature method (5), using phase-transfer catalysis for the dichlorocarbene addition (6). Spiropyrrolidine hydrochlorides (IX-XII) were synthesized from I according to Scheme I.

**Ethyl Bicyclo[3.2.1]octan-3′-yliden Cyanoacetate (II)**—A solution of I (16.6 g, 0.133 mol), ethyl cyanoacetate (15.12 g, 0.133 mol), ammonium acetate (2.10 g, 0.026 mol), and glacial acetic acid (6.8 mL) in benzene (50 mL) was refluxed for 24 h using a Dean-Stark water separator. The mixture was cooled, washed with water, and dried over anhydrous $Na_2SO_4$. The benzene was removed under reduced pressure, and the residue was distilled *in vacuo* to yield 29.2 g (89%) of a colorless liquid, bp 103°C (0.5 mm Hg). IR (neat film): $\nu$ 2220 (C≡N) and 1725 $cm^{-1}$ (C=O); $^1$H-NMR ($C_6D_6$): $\delta$ 4.00 (q, 2, $CH_2$), 2.10 and 1.20 (two m, 12, cyclic H), and 1.00 ppm (t, 3, $CH_3$).

*Anal.*—Calc. for $C_{13}H_{17}NO_2$: C, 71.2; H, 7.8; N, 6.4. Found: C, 70.9; H, 7.8; N, 6.3.

**3-Carboxybicyclo[3.2.1]octane-3-acetic Acid (III)**—To a solution of ester II (17.25 g, 0.078 mol) in ethanol (170 mL), a solution of KCN (13.36 g, 0.2 mol) in water (34 mL) was added. After stirring for 5 d, the solvent was removed at reduced pressure. The residue was heated at reflux for 48 h with concentrated HCl (134 mL). The mixture was diluted with an equal volume of water and cooled overnight. The precipitate was removed by filtration and washed with water. The crude product was dissolved in a boiling saturated solution of $KHCO_3$, decolorized with charcoal, filtered, and the product was precipitated by the addition of concentrated HCl to yield 11.8 g (71%) of III as a white solid, mp 156-157°C. IR (KBr): $\nu$ 3100-2500 (COOH) and 1725 and 1700 $cm^{-1}$ (COOH); $^1$H-NMR ($Me_2SO$-$d_6$): $\delta$ 12.00 (br s, 2, COOH), 2.40 (s, 2, $CH_2$—COOH), and 2.15 and 1.50-1.15 ppm (two m, 12, cyclic H).

*Anal.*—Calc. for $C_{11}H_{16}O_4$: C, 62.6; H, 7.5. Found: C, 62.3; H, 7.8.

**Bicyclo[3.2.1]octane-3-spiro-3′-succinic Anhydride (IV)**—Carboxylic acid III (7.0 g, 0.033 mol) and acetic anhydride (20 mL) were refluxed for 2 h. The mixture was concentrated to dryness and crystallized from petroleum ether to give 6.02 g (94%) of the anhydride IV as a white solid, mp 57°C. IR (KBr): $\nu$ 1860 and 1790 $cm^{-1}$ (C=O); $^1$H-NMR ($CDCl_3$, 250 MHz): $\delta$ 2.80 (s, 2, $CH_2$—CO), 2.34 (br s, 2, $C_{1(5)}H_X$), 2.18 (dd, 2, $J_{BA}$ = −14.34 Hz, $C_{2(4)}H_B$), 1.96 (dd, 2, $J_{B'A'}$ = −8.09 Hz, $C_{6(7)}H_{B'}$), 1.80 (dd, 2, $J_{AB}$ = −14.34 Hz, $C_{2(4)}H_A$), and 1.70 ppm (dd, 2, $J_{A'B'}$ = −8.09 Hz, $C_{6(7)}H_{A'}$).

*Anal.*—Calc. for $C_{11}H_{14}O_3$: C, 68.04; H, 7.21. Found: C, 68.31; H, 7.55.

***N*-Substituted Bicyclo[3.2.1]octane-3-spiro-3′-succinimides**—*General Procedure*—To a solution of anhydride IV (0.15 mol) in hot benzene (50 mL), the corresponding amine (0.15 mol) in benzene (30 mL) was added with vigorous stirring at 60-65°C. After standing for 0.5 h at 60-65°C, the mixture was cooled, and the precipitate was removed by filtration and washed with benzene. The carboxyamide was heated for 1 h at 220-225°C. After cooling, the residue was crystallized giving the corresponding succinimides.

*N-Benzyl Bicyclo[3.2.1]octane-3-spiro-3′-succinimide* (*V*)—Yield: 78%; mp 68-69°C (white prisms, absolute ethanol). IR ($CHBr_3$): $\nu$ 1740 and 1714 $cm^{-1}$ (C=O); $^1$H-NMR ($CCl_4$): $\delta$ 7.15 (s, 5, ArH), 4.40 (s, 2, $CH_2C_2H_5$), 2.40 (s, 2, $C_{4'}H_2$), and 2.20, 1.80, and 1.40 ppm (m, 12, cyclic H).

*Anal.*—Calc. for $C_{18}H_{21}NO_2$: C, 76.3; H, 7.4; N, 4.9. Found: C, 76.3; H, 7.7; N, 5.0.

*N-Cyclohexyl Bicyclo[3.2.1]octane-3-spiro-3′-succinimide* (*VI*)—Yield: 58%; mp 78-79°C (white prisms, absolute ethanol). IR ($CHBr_3$): $\nu$ 1775 and 1700 $cm^{-1}$ (C=O); $^1$H-NMR ($CDCl_3$): $\delta$ 3.90 (m, 1, CH—N), 2.50 (s, 2, $C_{4'}H_2$), and 2.20, 1.80 and 1.40 ppm (m, 22, cyclic H).

*Anal.*—Calc. for $C_{17}H_{25}NO_2$: C, 74.2; H, 9.1; N, 5.1. Found: C, 73.9; H, 9.3; N, 5.0.

*N-n-Butyl Bicyclo[3.2.1]octane-3-spiro-3′-succinimide* (*VII*)—Yield: 66%; mp 86-87°C (white prisms, absolute ethanol). IR ($CHBr_3$): $\nu$ 1770 and 1700 $cm^{-1}$ (C=O); $^1$H-NMR ($CCl_4$): $\delta$ 3.35 (t, 2, $CH_2$—N), 2.50 (s, 2, $C_{4'}H_2$), 2.25, 1.80, and 1.35 (m, 18, cyclic H), and 1.00 ppm (t, 3, $CH_3$).

*Anal.*—Calc. for $C_{15}H_{23}NO_2$: C, 72.3, H, 9.3; N, 5.6. Found: C, 72.6; H, 9.5; N, 5.6.

*N-β-Hydroxyethyl Bicyclo[3.2.1]octane-3-spiro-3′-succinimide* (*VIII*)—Yield: 39%; mp 98-99°C (white plates, absolute ethanol). IR ($CHBr_3$): $\nu$ 3450 (OH), and 1775 and 1700 $cm^{-1}$ (C=O); $^1$H-NMR ($CDCl_3$-$CCl_4$): $\delta$ 3.55 (s, 4, $CH_2$—OH and $CH_2$—N), 3.00 (s, 1, OH), 2.60 (s, 2, $C_{4'}H_2$), and 2.25, 1.80, and 1.40 ppm (m, 12, cyclic H).

*Anal.*—Calc. for $C_{13}H_{19}NO_3$: C, 65.8; H, 8.2; N, 5.9. Found: C, 65.6; H, 8.3; N, 5.7.

***N*-Substituted Bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine Hydrochlorides**—*General Procedure*—A solution of the corresponding succinimide (0.08 mol) in ether was slowly added with stirring to a suspension of lithium aluminum hydride (0.32 mol) in ether. The mixture was stirred overnight, decomposed, filtered, and dried over anhydrous $Na_2SO_4$. The solvent was removed at reduced pressure, and the pyrrolidine was distilled. Pyrrolidine hydrochlorides were formed by treatment of the bases with 3 M HCl. After concentration under reduced pressure, the hydrochloride was crystallized.

*N-Benzyl Bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine Hydrochloride* (*IX*)—Yield: 83%; mp 265-266°C (white prisms, isopropyl alcohol). IR ($CHBr_3$): $\nu$ 2500 (N—H), and 1450, 1435, and 1410 $cm^{-1}$ (aromatic); $^1$H-NMR ($CDCl_3$): $\delta$ 7.65-7.35 (m, 5, ArH), 4.30 (s, 2, $CH_2C_6H_5$), 3.65-3.00 (m, 4, $C_{2'}H_2$ and $C_{5'}H_2$), and 2.25, 1.65, and 1.50 ppm (m, 14, $C_{4'}H_2$ and cyclic H).

*Anal.*—Calc. for $C_{18}H_{26}ClN$: C, 74.1; H, 8.9; N, 4.8. Found: C, 73.8; H, 9.0; N, 5.0.

*N-Cyclohexyl Bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine Hydrochloride* (*X*)—Yield: 57%; mp 178-179°C (white prisms, acetone). IR ($CHBr_3$): $\nu$ 2500 $cm^{-1}$ (N—H); $^1$H-NMR ($CDCl_3$): $\delta$ 3.65-2.95 (m, 5, $C_{2'}H_2$, $C_{5'}H_2$, and CH—N), and 2.00, 1.70, and 1.40 ppm (m, 24, cyclic H).

*Anal.*—Calc. for $C_{17}H_{30}ClN$: C, 71.9; H, 10.6; N, 4.9. Found: C, 71.6; H, 10.3; N, 4.8.

*N-n-Butyl Bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine Hydrochloride* (*XI*)—Yield: 39%; mp 186-187°C (white prisms, acetone). IR ($CHBr_3$): $\nu$ 2500 $cm^{-1}$ (N—H); $^1$H-NMR ($CDCl_3$): $\delta$ 3.76-2.90 (m, 6, $C_{2'}H_2$, $C_{5'}H_2$, and $CH_2$—N), 2.25, 1.90, 1.70, and 1.40 [m, 18, $(CH_2)_2$ and cyclic H], and 1.00 ppm (t, 3, $CH_3$).

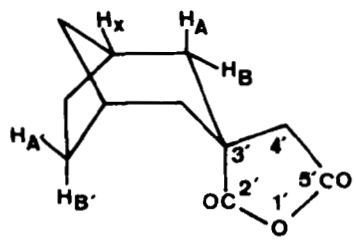
*Anal.*—Calc. for $C_{15}H_{28}ClN$: C, 69.9; H, 10.9; N, 5.4. Found: C, 69.6; H, 10.6; N, 5.1.

*N-β-Hydroxyethyl Bicyclo[3.2.1]octane-3-spiro-3′-pyrrolidine Hydrochloride* (*XII*)—Yield: 74%; mp 160-161°C (white prisms, absolute ethanol). IR ($CHBr_3$): $\nu$ 3280 (OH) and 2500 $cm^{-1}$ (N—H); $^1$H-NMR ($D_2O$): $\delta$ 3.60 (t, 2, $CH_2$—OH), 3.20 (m, 6, $CH_2$—N, $C_{2'}H_2$, and $C_{5'}H_2$), 2.00, 1.50, and 1.15 ppm (m, 14, $C_{4'}H_2$ and cyclic H).

*Anal.*—Calc. for $C_{13}H_{24}ClNO$: C, 63.5; H, 9.4; N, 5.7. Found: C, 63.8; H, 9.7; N, 5.4.

## RESULTS AND DISCUSSION

**Structural Assignment**—Due to the rigid structure of the bicyclo[3.2.1]-octane system, two different isomers (XIII and XIV) are possible. On the basis of the $^1$H-NMR spectrum of the anhydride IV, only the configuration shown (XIII) appears to be present.



From the 250-MHz $^1$H-NMR spectrum of anhydride IV, only one isomer is observed from the presence of only one singlet at $\delta$ 2.80 ppm for the $C_{4'}$-methylene group. The presence of two isomers should be evidenced by the

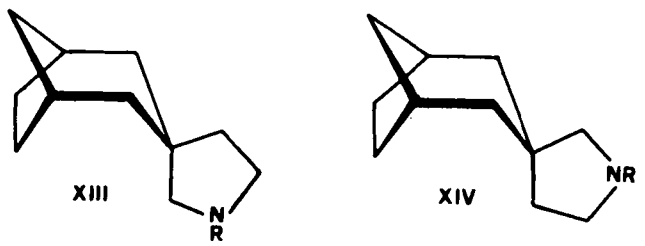
[1] All melting points were taken in open capillary tubes. Melting and boiling points are uncorrected. IR spectra were carried out on a Perkin-Elmer 577 spectrophotometer. $^1$H-NMR spectra were recorded in $CDCl_3$ solution at room temperature using Bruker WM-250 (250 MHz) and Varian EM 360-A (60 MHz) spectrometers ($Me_4Si$ as internal reference): (s) singlet; (d) doublet; (t) triplet; (m) multiplet; (br s) broad signal; (dd) doublet-doublet.

Table I—Inhibition of Growth of Influenza A Viruses by IX-XII and 1-Adamantanamine Hydrochloride

| Inhibitor[a] | Titration of Infection[b], Plaque-Forming Units/mL | Inhibition[c] |
|---|---|---|
| None | $2 \times 10^7$ | — |
| IX | $1 \times 10^6$ | −1.30 |
| X | $7 \times 10^5$ | −1.45 |
| XI | $1 \times 10^7$ | −0.30 |
| XII | $7 \times 10^6$ | −0.45 |
| 1-Adamantanamine hydrochloride | $2 \times 10^6$ | −1.00 |

[a] Inhibitors were administered at a concentration of 100 μM in an alcohol-water solution. [b] Plaque assay and primary isolation of influenza A viruses were carried out in an established line of canine kidney cells (MDCK), in the presence of trypsin (7). [c] Inhibition is expressed as the decimal logarithm of the quotient of plaque-forming units in the presence of inhibitor to that found in the absence of inhibitor: $\log_{10}$ [(pfu/mL of inhibitor)/(pfu/mL of control)].

appearance of two singlets, one each for the *endo* and *exo* $C_{4'}$-methylene groups. The same feature was observed in the NMR spectra of succinimides V-VIII.




Protons $H_AH_BH_{A'}H_{B'}H_X$ behave as two different ABX and A'B'X systems. The influence of the deshielding effect of the *endo* $C_{2'}$-carbonyl group on protons $H_B$ and $H_{B'}$ is shown by the downfield chemical shift of these protons at $\delta H_B = 2.18$ ppm and $\delta H_{B'} = 1.96$ ppm relative to those of the less-affected protons $H_A$ and $H_{A'}$, which appear upfield at $\delta$ 1.80 and 1.70 ppm, respectively. Assignation of $H_B$ and $H_{B'}$ has been done on the basis of the observable geminal coupling constants $J_{BA} = -14.34$ Hz and $J_{B'A'} = -8.09$ Hz, which are in agreement with reported values for cyclohexane and cyclopentane, respectively (8).

X-ray diffraction data of succinimide VI (9) conclusively confirm that the $C_{2'}$-carbonyl group is attached on the *endo*-position. In this case the spirocyclohexane ring appears to adopt a boat conformation to avoid the steric interaction between the $C_{2'}$-carbonyl group and the $C_{6(7)}$-methylene groups.

**Biology**—According to results shown in Table I, IX-XII, especially the *N*-benzyl and *N*-cyclohexyl derivatives (IX and X), show antiviral activity against influenza A viruses similar to or greater than that shown by 1-adamantanamine hydrochloride.

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# Distribution and Elimination of Polymethyl Methacrylate Nanoparticles After Peroral Administration to Rats


**MARIJKE NEFZGER *, JÖRG KREUTER ‡x, ROLF VOGES §, EKKE LIEHL *, and RUDOLF CZOK ***



Received March 7, 1983 from the **Sandoz Forschungsinstitut GmbH, Brunner Strasse 59, A 1235 Vienna, Austria*, the ‡*Department of Pharmaceutics, Swiss Federal Institute of Technology, Clausiusstrasse 25, CH 8092 Zurich, Switzerland*, and the §*Pharmaceutical Division, Sandoz Ltd., CH 4002 Basle, Switzerland.* Accepted for publication September 13, 1983.



**Abstract** □ Polymethyl [1-$^{14}$C]methacrylate nanoparticles were administered orally to bile cannulated rats. Ten to fifteen percent of the administered radioactivity was absorbed and found in the bile and urine. Within 48 h, 94 97% of the absorbed radioactivity had been eliminated from the body. After 8 d, the highest residual radioactivity was found in the bone marrow, fatty renal tissue, stomach, liver, and lymph nodes.



**Keyphrases** □ Polymethyl methacrylate—peroral administration, distribution, elimination, rats □ Peroral administration—polymethyl methacrylate, elimination and distribution, rats


A possible pathway of absorption is the uptake of colloidal particulate materials by the GI tract in liquid (pinocytosis) or solid (endocytosis) form. This uptake pathway was suggested for fat absorption by Frazer (1, 2). The uptake of corn starch and some other particulate materials by endocytosis was extensively studied by Volkheimer (3). No uptake of polymethyl methacrylate particles (labeled with a fluorescent dye and ranging in size from 10 nm–1.2 μm) was observed by Juhlin (4). This may, however, be due to leakage of the label or to the small size of the particles, which prevented optical observation. For this reason, $^{14}$C-labeled polymethyl methacrylate nanoparticles of a mean size of 130 nm were employed in this study. These particles were shown to be taken up by the reticuloendothelial system after intravenous administration (5). After subcutaneous administration, the particles stayed at the injection site for about 200 d. After this time, the beginning of redistribution and elimination was observed (6).

Oral administration of these nanoparticles seems to be of