PMR and ^{13}C-NMR Spectroscopy of Tropane and *N*-Substituted Nortropane Spirohydantoins

G. G. TRIGO ˣ, M. MARTÍNEZ, and E. GÁLVEZ

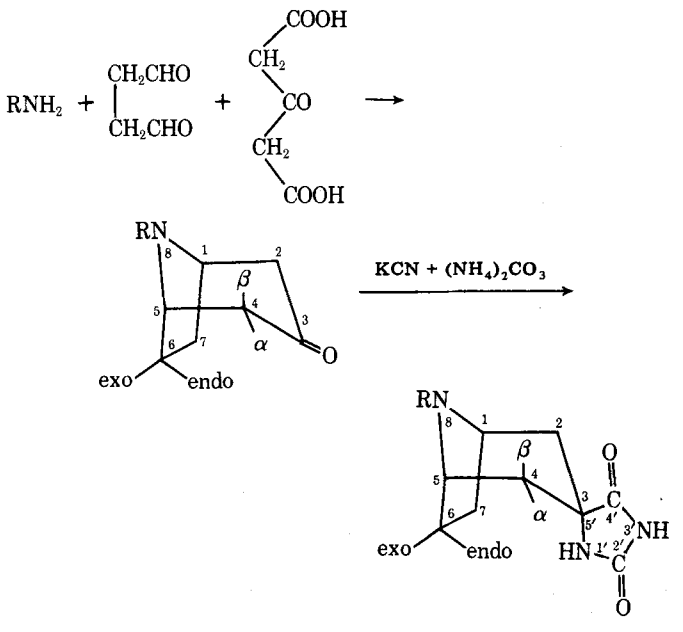
Received April 9, 1980, from the *Department of Organic and Pharmaceutical Chemistry, School of Pharmacy, Universidad Complutense, Madrid-3, Spain.* Accepted for publication July 17, 1980.

Abstract □ The preferred conformations of a pharmacologically interesting series of *N*-substituted nortropane spirohydantoins were determined from PMR and ^{13}C-NMR data to permit a structure–activity relationship study.

Keyphrases □ Tropane and *N*-substituted nortropane spirohydantoins—PMR and ^{13}C-NMR spectra, determination of preferred conformation □ ^{13}C-NMR spectroscopy—tropane and *N*-substituted nortropane spirohydantoins, determination of preferred conformation

A series of tropane and nortropane 3-spiro-5′-hydantoins, synthesized previously following the method outlined in Scheme I (1), showed anticonvulsant activity against pentylenetetrazol-induced convulsions in mice (2) and antiarrhythmic activity in rabbits previously treated with ouabain (2). Compound V is as active as procainamide in this assay. It showed an inhibitory effect on sodium transport in frog skin. As with diphenylhydantoins, it did not alter the initial inhibitory phase in the action of isoproterenol on frog skin, but it did alter subsequent stimulation (3). Furthermore, several compounds showed a local anti-inflammatory action comparable to that of indomethacin and greater than that of phenylbutazone (4).

These observations prompted the PMR and ^{13}C-NMR study of these compounds to determine their preferred conformation in solution with the objective of establishing a structure–activity relationship.



I: R = H
II: R = CH_3
III: R = C_2H_5
IV: R = *n*-C_3H_7
V: R = *iso*-C_3H_7
VI: R = *n*-C_4H_9
VII: R = CH_2CH_2OH
VIII: R = C_6H_5
IX: R = $CH_2C_6H_5$
X: R = $CH_2CH_2C_6H_5$

Scheme I

EXPERIMENTAL

The synthesis and purification of the compounds were described previously (1). The PMR spectra were recorded at 100 MHz[1] at room temperature. The ^{13}C-NMR spectra were determined at 20 MHz[2] and at 25.2 MHz[1] in the Fourier transform mode. The spectra were obtained at room temperature by using the deuterium resonance of the solvent (deuterium oxide) as the lock signal. Dioxane (67.4 ppm downfield relative to tetramethylsilane) was used as the reference. Both broad band decoupled and single-frequency off-resonance decoupling spectra were determined.

RESULTS AND DISCUSSION

PMR Spectra—The PMR spectra of all of the spirohydantoins (Table I) displayed a fairly symmetrical two-proton band at ~4.0 ppm, which was assigned to the C-1 and C-5 hydrogens; the widths at half of the maximum height ($W_{1/2}$) of these signals were taken to measure the extent of spin coupling (5). Coupling between the C-1 and C-5 methine protons and the C-6 and C-7 methylene protons was assumed to be constant throughout the series.

The $W_{1/2}$ value for the C-1 and C-5 hydrogen signals (Table II) fell in the range of 9–11 Hz, which corresponds to a chair conformation of the piperidine ring, since the corresponding C-1 and C-5 hydrogen signals for a boat conformation are ~18 Hz (5). The chair conformation of the piperidine ring is corroborated by the values of the coupling constants $JH_{2,4\alpha}$–$H_{1,5}$ and $JH_{2,4\beta}$–$H_{1,5}$ (Table II). Furthermore, $JH_{2,4\beta}$–$H_{1,5}$ is greater than $JH_{2,4\alpha}$–$H_{1,5}$, and, therefore, the dihedral angle $H_{2,4\alpha}$–C–C–$H_{1,5}$ is greater than $H_{2,4\beta}$–C–C–$H_{1,5}$ due to a flattening of the piperidine ring.

In all of the spirohydantoins studied, there was a difference of ~0.6 ppm between the δ values corresponding to H-2α and H-4α and H-2β and H-4β (Table I). This difference was produced by the field effect due

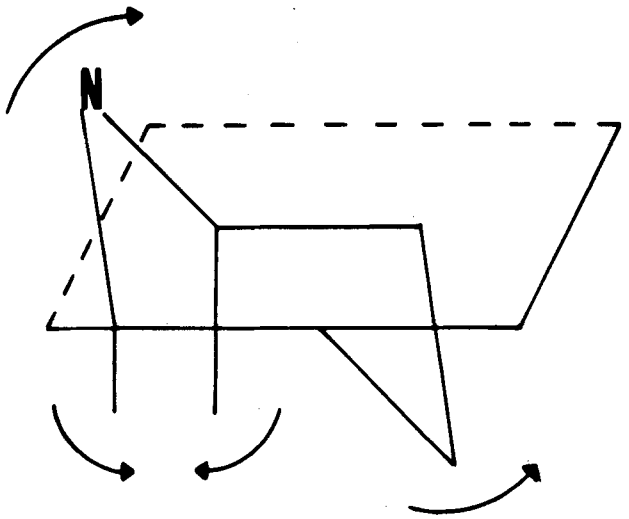


Figure 1—*Drawing showing piperidine linkage.*

[1] Varian XL 100 spectrometer.
[2] Varian CFT 20 spectrometer.

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Table I—Chemical Shifts [a] of Hydrochlorides of I–X in Dimethyl Sulfoxide (Tetramethylsilane as Internal Reference)

Group	I	II[b]	III	IV	V	VI	VII	VIII	IX	X
$H_{2,4\alpha}$	1.83 (a)	2.14 (a)	1.95 (a)	1.93 (a)	1.9 (a)	1.84 (a)	1.94 (a)	1.42 (a)	1.93 (a)	2.0 (a)
$H_{2,4\beta}$	2.40 (a)	2.61 (a)	2.46 (a)	2.46 (a)	2.4 (a)	2.45 (a)	2.6 (a)	2.2 (a)	2.56 (a)	2.6 (a)
$H_{1,5}$	4.0 (b)	4.07 (b)	4.03 (b)	4.10 (b)	4.10 (b)	3.9 (b)	3.81 (b)	4.26 (b)	4.22 (b)	4.15 (b)
$H_{6,7}$	2.13 (b)	2.37 (c)	2.2 (b)	2.18 (b)	2.18 (b)	2.16 (b)	2.20 (b)	2.0 (b)	2.32 (b)	2.3 (b)
$CH\alpha$		2.87 (d)	3.06 (e)	2.92 (f)[c]	4.1 (c)	2.91 (f)	4.12 (b)		3.82 (b)	3.2
$CH\beta$			1.26 (f)	1.72 (g)	1.34 (h)	1.64 (i)[c]	3.08 (b)			3.2
$CH\gamma$				0.9 (f)		1.34 (g)				
$CH\delta$						0.92 (f)				
Aromatic								6.5–6.8 (c)	7.4–7.6 (c)	7.4 (d)
								7.0–7.2 (c)	7.6–7.8 (c)	

[a] a = doublet of doublets, b = wide singlet, c = multiplet, d = singlet, e = quartet, f = triplet, g = sextuplet, h = doublet, and i = quintuplet. [b] Spectra were recorded in deuterium oxide as solvent. [c] Not resolved.

Table II—Coupling Constants of Hydrochlorides of I–X in Dimethyl Sulfoxide (Hertz Values, Tetramethylsilane as Internal Reference)

Identification	I[a]	II	III	IV	V	VI	VII	VIII	IX	X
$JH_{2,4\alpha}$–$H_{2,4\beta}$	14	16	16	15	16	16	16	14	14	14
$JH_{2,4\beta}$–$H_{1,5}$	3	3	3	~3	~3	3	~3	3	3	~3
$JH_{2,4\alpha}$–$H_{1,5}$	<1	<1	1	~1	~2	1	~2	1	<1	~2
$JH_{1,5}$ ($W_{1/2}$)	9	9	10	9	10	9	10	9	9	11
JH_{α}–H_{β}			8		6	8				
JH_{β}–H_{γ}				8		8				
JH_{γ}–H_{δ}						8				

[a] Spectra were recorded in deuterium oxide.

to the magnetic anisotropy of the C-4′ carbonyl group, which occupies an equatorial position with regard to the piperidine ring (6, 7). This arrangement was confirmed in II by X-ray diffraction data (8).

In VIII, the signals corresponding to H-2 and H-4 were shifted toward a higher field ($\Delta\delta$ = 0.4 ppm) with respect to the other compounds of the series, as expected. However, the signal corresponding to H-1 and H-5 was deshielded ~0.3 ppm with respect to the same signal of the other compounds (Table I). This result was attributed to the field effect due to the anisotropy of the *N*-equatorial phenyl group when this group adopts the more favored conformation, which has the plane of the aromatic ring at right angles to a plane passing through the nitrogen and C-4 of the piperidine ring (9).

^{13}C-NMR Spectra—In all cases, noise-decoupled and single-frequency off-resonance decoupling spectra were obtained.

The ^{13}C-NMR data of I, II, and X are summarized in Table III. Assignments of the carbon resonances were made by the multiplicity of signals in the single-frequency off-resonance decoupling spectra, the peak intensity of the noise-decoupled spectra, the literature data (10–14), and conventional techniques.

In all cases, the δ values of the C-2 and C-4 signals (Table III) clearly indicated the equatorial position of the radical attached to the piperidine nitrogen atom since, for an *N*-axial position, a great interaction would be apparent at C-2 and C-4 due to the steric compression between the axial C-2 and C-4 protons and the axial *N*-group, giving place to a shielding of ~20 ppm for the C-2 and C-4 atoms (13).

By comparing the C-6 and C-7 signals of I with the same carbon signals of II and X (Table III), an effect of the *N*-methyl and *N*-phenethyl groups of 1.2 and 1.7 ppm, respectively, can be deduced. The same γ-effect for the couple nortropane–tropane has a value of 3.4 ppm (10); an analogous γ-effect for 7-methylnorbornane has a value of 3.8 ppm (15). The smaller γ-effect in II and X, with respect to tropane and 7-methylnorbornane, as well as the mentioned flattening of the piperidine ring deduced in the PMR data, can be explained as follows. The linkage of the 2,6-positions of piperidine by a dimethylene bridge causes the 2,6-axial bonds to move together and the 3,5-axial bonds to diverge, while the nitrogen moves away from and C-4 moves nearer to the mean plane of the ring (Fig. 1) (16, 17). These distortions in I–X are increased by the steric compression between the ethylene bridge and the hydantoin ring.

The δ values of C-2 and C-4 in I, II, and X are in agreement with a chair conformation in the piperidine ring, with an additional shielding due to the introduction of a γ-*gauche* interaction at these carbon atoms with the NH axial proton (13). The signals of C-1 and C-5 also are in agreement with a chair conformation of the piperidine ring since the shielding effect is not observed as a consequence of eclipsing between the C-1 and C-5 and the C-2 and C-4 hydrogen atoms in the boat conformer (13).

Table III—^{13}C-Chemical Shifts for Hydrochlorides of I, II, and X in Deuterium Oxide

Carbon Position	Multiplicity[a]	I	II	X[b]
1, 5	d	54.33	63.20	61.35
2, 4	t	37.99	40.08	39.27
3	s	59.88	59.75	59.20
6, 7	t	25.29	24.12	23.63
N-CH_3	q	—	40.88	—
N-CH_2CH_2	t	—	—	53.30
N-CH_2CH_2	t	—	—	31.27
C-2′	s	159.80	160.80	159.40
C-4′	s	180.60	181.09	179.90

[a] Signal multiplicity obtained from single-frequency off-resonance decoupling spectra: s = singlet, d = doublet, t = triplet, and q = quartet. [b] Aromatic: C-1, 137.3; C-2 and C-6**, 129.61; C-3 and C-5**, 132.0; and C-4, 128.24 (** Values may be interchanged).

CONCLUSION

The results obtained by X-ray diffraction of II are in good agreement with the PMR and ^{13}C-NMR conclusions. From the X-ray diffraction studies (8), it was deduced that: (*a*) the piperidine ring has a flattened chair conformation; (*b*) the piperidine nitrogen atom shows a pyramidal geometry, and the methyl group attached to it is in an axial position; and (*c*) the dihedral angle $C_{6,7}$–$C_{1,5}$–N_8 has a smaller value compared to the same dihedral angle in other tropane derivatives such as pseudotropine (17) and α-chlorotropane monohydrate (18), which both have the *N*-methyl group in the equatorial position.

The main reason for the *N*-methyl axial position in the crystalline state of II (8) probably is to make possible the intermolecular hydrogen bonding between the N-3′ hydrogen and N-8. When II as its hydrochloride is dissolved in deuterium oxide, the *N*-methyl group occupies an equatorial position to avoid the diaxial interaction with the axial C-2 and C-4 hydrogen atoms; by consequence, the dihedral angle $C_{6,7}$–$C_{1,5}$–N_8 is increased to decrease the steric compression between the C-6 and C-7 *exo*-hydrogen atoms and the *N*-equatorial group.

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Two-Dimensional TLC Analysis of Ginsenosides from Root of Dwarf Ginseng (*Panax trifolius* L.) Araliaceae

TAIKWANG M. LEE and ARA DER MARDEROSIAN ˣ

Received May 15, 1980, from the *Department of Biological Sciences, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104.* Accepted for publication July 3, 1980.

Abstract □ A procedure is reported for the two-dimensional TLC separation of ginsenosides from the root of *Panax trifolius* L. The solvent systems and spray reagents described are useful for the identification of ginsenosides in various species of *Panax*. The results of the separation and identification with controls of at least four ginsenosides from the root of *P. trifolius* L. are reported. The total percentage of ginsenosides was 0.0061%.

Keyphrases □ Ginsenosides—two-dimensional TLC analysis from root of dwarf ginseng □ *Panax trifolius* L.—two-dimensional TLC analysis of ginsenosides from root □ Ginseng, dwarf—two-dimensional TLC analysis of ginsenosides from root

Dwarf ginseng[1] is a member of the ginseng family (Araliaceae) and is distributed from southern Canada to the northern United States (1). The plant is small and delicate and has a whorl of three stalked leaves, each of which is divided into three stalkless leaflets. The plant has a small round umbel of white flowers, which develop into greenish-yellow fruits, and a round tuberous root, which grows deep in the ground.

This report describes the usefulness of two-dimensional TLC for the semimicro separation of the ginsenosides isolated from the root of dwarf ginseng (*Panax trifolius* L.).

EXPERIMENTAL

Plant Material—Specimens of wild dwarf ginseng (*P. trifolius* L.) were collected on May 4, 1979, from the Tyler Arboretum, Delaware County, Pa.

Extraction Procedure—Ground, freeze-dried roots (18.6 g) were extracted with chloroform (150 ml) to remove pigments and lipids. The marc was air dried and extracted again with methanol (150 ml). Methanol-washed silica gel (10.0 g) was mixed well with the methanolic extract and filtered to remove impurities (2). The filtrate was combined with the methanolic silica gel washings and concentrated to 5 ml. Ten milliliters of water was added to 50 ml of the methanolic extract and then reextracted three times with water-saturated 1-butanol (60 ml total). The three butanol layers were combined and concentrated to yield the crude saponin extract (20 mg), and this extract was examined by two-dimensional TLC.

Two-Dimensional TLC—*Adsorbent*—Precoated silica gel 60 F-254 silanized TLC plates[2] were used (20 × 20 cm, 250-μm layer).

Eluent Systems—Solvents were analytical grade and were used as received[3]. Eluent A was chloroform–methanol–ethyl acetate–butanol–water (4:4:8:1:2, lower phase). Eluent B was chloroform–butanol–methanol–water (4:8:3:4, lower phase). Eluent C was chloroform–methanol–water (13:7:2, lower phase).

Detection Reagent—A solution of 1 ml of *p*-anisaldehyde and 1 ml of concentrated sulfuric acid diluted to 100 ml with methanol was prepared fresh daily. Plates were heated at 130° for 15 min following spraying (3).

Standard Solutions—Standard solutions consisted of ginsenosides[4] Ro, Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, and Rg_2 dissolved in methanol at a concentration of 1 mg/ml. All stock solutions were kept refrigerated (3).

Procedure—The concentrated crude saponins were dissolved in methanol (1 ml), and 20 μl of this solution was spotted on the bottom left corner of the plates 2.0 cm from each side. The standard solutions of 5 μl of each control ginsenoside were spotted separately 0.8 cm from each other on the bottom right and upper left, exactly 10.0 cm from the crude saponin spot. Two-dimensional TLC plates were prepared as shown in Fig. 1.

The plates were developed separately in one direction in Eluents A and B for 10 cm. After air drying for 10 min, the plates were run in the second direction using Eluent C perpendicular to the first direction for a distance of 10 cm. The R_f values were calculated on the basis of a solvent front of 10.0 cm above the origin, and the relative R_f values were calculated with the ginsenoside Rf as the standard.

Quantitative Analysis—Quantitation of the saponins of *P. trifolius* L. was performed using the spectrodensitometric procedure described previously (3). A 250-mg portion of the root was extracted, and 3.5 ml of the final 1-butanol solution was evaporated to dryness. The residue was

[1] This is the first report of continuing research on *Panax trifolius* L. All plant parts of dwarf ginseng are currently being studied in detail.

[2] Catalog No. 5601, EM Laboratories, Elmsford, NY 10523.
[3] Fisher Scientific Co., Fair Lawn, NJ 07410.
[4] Nine standard ginsenosides were obtained from Dr. J. Shoji, Showa University, Tokyo, Japan.

0022-3549/81/0100-0089$01.00/0