Synthesis, Structural, Conformational and Biochemical Study of some 3β-Acyloxytropan-3α-carboxylic Acid Hydrochlorides

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A series of 3β-acyloxytropan-3α-carboxylic acid hydrochlorides have been synthesized and studied by ^1H and ^{13}C nmr spectroscopy, and the crystal structure of 3β-(3,4,5-trimethoxybenzoyloxy)tropan-3α-carboxylic acid hydrochloride **4c** has been determined by X-ray diffraction. The compounds studied display in methanol-d_4 the same preferred conformation. The pyrrolidine and piperidine rings adopt a flattened N8 envelope and distorted chair conformation; puckered at N8 and flattened at C3 respectively with the *N*-substituent in equatorial position with respect to the piperidine ring. In all cases, there is only one mode (axial) of proton uptake at the piperidine nitrogen atom. These results are in close agreement with that found for compound **4c** in the crystalline state. The inhibitory ability of the title compounds upon ^3H-GABA binding to sinaptosomal brain membranes is also reported.

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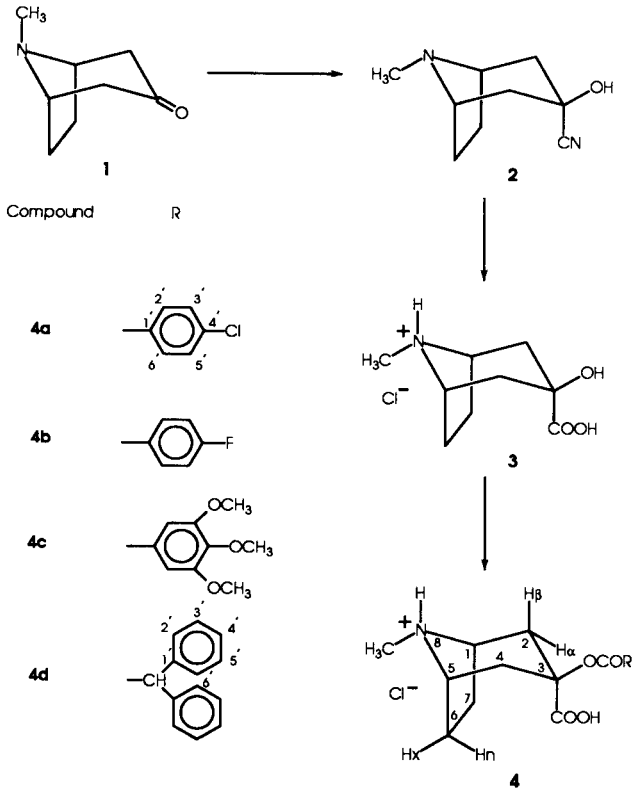
Introduction.

Previously [1] we have studied the synthesis, the ir, ^1H and ^{13}C nmr studies and the crystal structure of several esters derived from 3β-hydroxytropan-3α-carboxylic acid as potential new $GABA_B$ receptor antagonists. In the same line we report in this paper the synthesis and structural analysis with the aid of ^1H and ^{13}C nmr spectroscopy of a series of 3β-acyloxytropan-3α-carboxylic acids in which the γ-aminobutyric acid (GABA) skeleton is included (Scheme I) with the objective to determine their preferred conformation both in solution and in the solid state, the crystal structure of compound **4c** has also been determined. The biochemical study of the title compounds has been carried out.

Scheme I



Results and Discussion.

Description of the Structure of Compound **4c**.

The main crystallographic data and the structure determination conditions are given in Table 1 [2-7], Table 2 shows the atomic parameters and Tables 3 and 4 show bond lengths, bond and torsion angles respectively. Several significant torsion angles in which hydrogen atoms are involved are also given.

Figure 1 [8] shows a view of the molecule with the labelled atomic numbering.

The bicyclic system adopts a chair-envelope conformation with the trimethoxybenzoyloxy group in an equatorial position. However, the chair is puckered at the N2 atom and very flattened at the disubstituted C5 atom, both being 0.91(1) and 0.36(1) Å, respectively, from the plane defined by the remaining four atoms; the conformation is therefore almost intermediate between a chair and an envelope, as shown by the ring puckering coordinates [9]:

Table 1

Experimental Data and Structure Refinement Procedures

Crystal data	
Formula	$C_{19}H_{26}NO_7Cl$
Crystal size (mm)	0.67 x 0.20 x 0.20
Symmetry	Orthorhombic, Pbca
Unit cell determination:	Least-squares fit from 50 reflections (θ<20°)
Unit cell dimensions	26.644(3), 12.476(1), 12.877(1) 90.0, 90.0, 90.0
Packing: V(A^3), z	4280.3(5), 8
Dc($g \cdot cm^{-3}$),M,F(000)	1.2906, 415.870, 1760
$\mu(cm^{-1})$	2.123
Experimental data	
Technique	Four circle diffractometer: Philips Pw1100. Bisecting geometry Graphite oriented monochromator: MoKα $\omega/2\theta$ scans, scan width: 1.2°θ Detector apertures 1 x 1, up θmax •28°
Number of reflections:	
Measured	5138
Observed	2084 (3σ(I) criterion)
Max-min transmission factors:	1.226, 0.721 (2)
Solution and refinement	
Solution	Direct methods and Fourier synthesis
Refinement	L.S. on FObs with 1 block
H atoms	Difference synthesis
w-scheme	Emperical as to give no trends in <wΔF> vs.<Fobs> and sinθ/λ>
Max thermal value	U22 (C28)= 0.43 (3)
Final ΔF peaks	0.4e/A^3
Final R and Rw	0.086, 0.061
Computer and programs	Vax 6410, Multan80 [3], X-ray System [4], Pesos [5], Parst [6]
Scattering factors	Int. Tables for X-ray Crystallography [7]
Anomalous dispersion	Int. Tables for X-ray Crystallography

Table 2

Atomic parameters for $C_{19}H_{26}NO_7Cl$

Coordinates and thermal parameters as Ueq=(1/3)•Sum(Uij•ai**aj**ai•aj•cos(ai,aj))•10**4

Atom	x	y	z	Ueq
Cl1	0.030186(7)	-0.00602(14)	0.37908(14)	390(5)
O12	0.24870(20)	0.28400(41)	-0.00066(38)	453(18)
O13	0.31757(19)	0.36395(40)	0.05924(39)	391(17)
O14	0.34003(16)	0.19481(39)	0.17616(34)	336(16)
O16	0.35816(18)	0.14918(47)	0.01144(40)	492(19)
O23	0.55279(21)	0.11348(59)	0.06876(53)	760(28)
O25	0.55815(21)	0.03912(54)	0.26162(57)	694(26)
O27	0.47713(20)	0.02115(78)	0.38040(53)	1033(35)
N2	0.21212(21)	0.12423(41)	0.29130(42)	276(18)
C3	0.20956(27)	0.09143(58)	0.17791(58)	353(24)
C4	0.26126(25)	0.10360(53)	0.13318(60)	304(22)
C5	0.28737(23)	0.21269(52)	0.15243(48)	250(20)
C6	0.26855(26)	0.26992(59)	0.25116(57)	294(23)
C7	0.21445(26)	0.24588(53)	0.28063(53)	294(22)
C8	0.17383(29)	0.26959(63)	0.19962(65)	418(28)
C9	0.16961(29)	0.16741(61)	0.13443(66)	384(25)
C10	0.16848(33)	0.08517(77)	0.35601(70)	485(31)
C11	0.28284(26)	0.28947(58)	0.06003(49)	303(23)
C15	0.37045(26)	0.15856(60)	0.10061(55)	344(24)
C17	0.41952(26)	0.12994(62)	0.14343(55)	391(26)
C18	0.46249(32)	0.13805(74)	0.08177(63)	462(30)
C19	0.50803(28)	0.10845(67)	0.12160(75)	494(29)
C20	0.51241(29)	0.06814(74)	0.22186(70)	543(33)
C21	0.46988(31)	0.06090(81)	0.28350(69)	612(35)
C22	0.42299(31)	0.09201(77)	0.24340(72)	536(32)
C24	0.55262(44)	0.15924(128)	-0.03215(101)	895(55)
C26	0.56986(57)	-0.07199(138)	0.25296(129)	991(70)
C28	0.43470(41)	0.00961(229)	0.44576(115)	1809(119)

Table 2 (continued)

Atomic parameters for $C_{19}H_{26}NO_7Cl$

Thermal parameters as exp[-2•pi**2• Sum(Uij•ai**aj**hi•hj) •10**4]

Atom	U11	U22	U33	U12	U13	U23
Cl1	484(9)	281(8)	404(9)	47(9)	-36(9)	-37(10)
O12	493(29)	495(35)	371(28)	-101(29)	-138(30)	128(27)
O13	434(30)	355(29)	384(30)	-91(26)	-53(25)	90(26)
O14	295(25)	442(30)	270(26)	59(23)	-37(22)	11(24)
O16	467(30)	688(39)	322(30)	143(29)	-52(26)	-34(30)
O23	360(34)	1154(58)	765(48)	2(37)	134(32)	180(44)
O25	311(33)	885(49)	886(50)	97(32)	-63(32)	242(42)
O27	382(34)	2080(91)	638(42)	113(48)	-89(33)	594(59)
N2	315(32)	208(28)	306(32)	-59(25)	-19(27)	29(26)
C3	396(44)	265(37)	399(44)	-22(35)	-56(36)	-56 (34)
C4	327(40)	245(35)	339(42)	42(30)	-43(34)	31(37)
C5	265(35)	289(35)	197(33)	25(30)	-77(28)	-7(29)
C6	367(40)	272(40)	244(38)	11(34)	-64(32)	-23(34)
C7	363(38)	274(37)	245(39)	109(33)	-10(31)	-28(32)
C8	443(48)	376(48)	433(48)	186(38)	-128(39)	-85(35)
C9	356(42)	364(42)	432(46)	8(34)	-132(37)	-112(39)
C10	436(48)	592(59)	427(52)	-109(45)	116(41)	-25(46)
C11	361(40)	397(44)	152(33)	62(37)	-7(31)	-4(33)
C15	357(40)	403(44)	272(41)	17(36)	-63(33)	46(35)
C17	299(39)	525(49)	349(46)	24(35)	-3(34)	128(41)
C18	490(53)	543(55)	353(45)	53(44)	-34(39)	106(41)
C19	311(42)	600(53)	572(53)	31(39)	34(43)	42(52)
C20	258(43)	782(65)	591(63)	66(42)	-89(41)	137(53)
C21	403(50)	928(73)	505(58)	31(47)	-91(44)	235(54)
C22	295(45)	788(65)	525(55)	83(47)	-96(41)	126(53)
C24	622(75)	1252(117)	812(91)	47(78)	152(64)	370(88)
C26	725(95)	1132(127)	1116(137)	219(91)	-178(89)	145(108)
C28	374(67)	4263(338)	788(90)	228(124)	-45(66)	1203(164)

Table 2 (continued)

Atomic parameters for $C_{19}H_{26}NO_7Cl$

Coordinates and thermal parameters as exp[-8•pi**2•U•(sin(theta)/lambda)**2 •10**3]

Atom	x	y	z	U
H13	0.322(3)	0.415(7)	0.000(7)	35(0)
H2	0.245(3)	0.085(6)	0.334(6)	35(0)
H3	0.200(3)	0.020(7)	0.176(6)	35(0)
H41	0.260(3)	0.088(7)	0.053(6)	35(0)
H42	0.283(3)	0.053(7)	0.164(7)	35(0)
H61	0.267(3)	0.347(7)	0.237(7)	35(0)
H62	0.291(3)	0.256(7)	0.307(7)	35(0)
H7	0.207(3)	0.276(6)	0.350(6)	35(0)
H81	0.139(3)	0.295(6)	0.238(7)	35(0)
H82	0.188(3)	0.327(7)	0.151(6)	35(0)
H91	0.172(3)	0.172(7)	0.051(7)	35(0)
H92	0.135(3)	0.138(7)	0.140(7)	35(0)
H101	0.135(3)	0.107(7)	0.323(7)	35(0)
H102	0.162(3)	0.110(7)	0.425(7)	35(0)
H103	0.168(3)	-0.002(7)	0.342(6)	35(0)
H18	0.457(3)	0.165(7)	0.020(7)	35(0)
H22	0.389(3)	0.087(6)	0.283(6)	35(0)
H241	0.537(3)	0.231(7)	-0.035(7)	35(0)
H242	0.526(3)	0.141(8)	-0.069(7)	35(0)
H243	0.593(3)	0.137(7)	-0.049(6)	35(0)
H261	0.567(4)	-0.099(9)	0.195(7)	35(0)
H262	0.551(4)	-0.093(9)	0.295(8)	35(0)
H263	0.605(3)	-0.082(6)	0.308(6)	35(0)
H281	0.400(3)	-0.026(7)	0.404(6)	35(0)
H282	0.413(4)	0.053(8)	0.451(8)	35(0)
H283	0.450(3)	0.004(8)	0.499(7)	35(0)

Table 3

Bond Distances (Å)

O12	-	C11	1.20(1)
O13	-	C11	1.31(1)
O14	-	C5	1.45(1)
O14	-	C15	1.34(1)
O16	-	C15	1.20(1)
O23	-	C19	1.37(1)
O23	-	C24	1.42(1)
O25	-	C20	1.37(1)
O25	-	C26	1.42(2)
O27	-	C21	1.36(1)
O27	-	C28	1.42(1)
N2	-	C3	1.52(1)
N2	-	C7	1.52(1)
N2	-	C10	1.51(1)
C3	-	C4	1.50(1)
C3	-	C9	1.53(1)
C4	-	C5	1.55(1)
C5	-	C6	1.54(1)
C5	-	C11	1.53(1)
C6	-	C7	1.52(1)
C7	-	C8	1.53(1)
C8	-	C9	1.53(1)
C15	-	C17	1.46(1)
C17	-	C18	1.49(1)
C17	-	C22	1.37(1)
C18	-	C19	1.37(1)
C19	-	C20	1.39(1)
C20	-	C21	1.39(1)
C21	-	C22	1.41(1)

Table 3 (continued)

Bond Angle (°)

C5	-	O14	-	C15	118.8(5)
C19	-	O23	-	C24	118.0(7)
C20	-	O25	-	C26	115.0(9)
C21	-	O27	-	C28	118.0(7)
C7	-	N2	-	C10	113.7(5)
C3	-	N2	-	C10	114.1(5)
C3	-	N2	-	C7	100.6(5)
N2	-	C3	-	C9	102.5(6)
N2	-	C3	-	C4	107.5(6)
C4	-	C3	-	C9	115.8(6)
C3	-	C4	-	C5	116.1(6)
O14	-	C5	-	C4	109.4(5)
C4	-	C5	-	C11	113.0(5)
C4	-	C5	-	C6	113.1(5)
O14	-	C5	-	C11	109.6(5)
O14	-	C5	-	C6	102.2(5)
C6	-	C5	-	C11	109.0(5)
C5	-	C6	-	C7	115.0(6)
N2	-	C7	-	C6	104.9(5)
C6	-	C7	-	C8	117.5(6)
N2	-	C7	-	C8	103.0(5)
C7	-	C8	-	C9	105.3(6)
C3	-	C9	-	C8	105.3(6)
O13	-	C11	-	C5	113.2(6)
O12	-	C11	-	C5	121.9(6)
O12	-	C11	-	O13	124.7(6)
O14	-	C15	-	O16	124.1(6)
O16	-	C15	-	C17	125.5(7)
O14	-	C15	-	C17	110.4(6)
C15	-	C17	-	C22	119.8(7)
C15	-	C17	-	C18	120.0(7)
C18	-	C17	-	C22	120.1(7)
C17	-	C18	-	C19	119.6(8)
O23	-	C19	-	C18	124.9(8)
C18	-	C19	-	C20	121.3(7)
O23	-	C19	-	C20	113.8(7)
O25	-	C20	-	C19	121.1(7)
C19	-	C20	-	C21	119.1(8)
O25	-	C20	-	C21	119.7(8)
O27	-	C21	-	C20	115.7(7)
C20	-	C21	-	C22	119.9(8)
O27	-	C21	-	C22	124.4(8)
C17	-	C22	-	C21	119.9(8)

Table 3 (continued)

Selected torsion angles (°)

C5	-O14	-C15	-C17	-171(1)
C5	-O14	-C15	-O16	7(1)
C15	-O14	-C5	-C11	-56(1)
C24	-O23	-C19	-C18	4(1)
C26	-O25	-C20	-C19	-96(1)

C28	-O27	-C21	-C20	-178(1)
O14	-C5	-C11	-O13	-36(7)
O14	-C5	-C11	-O12	148(1)
O16	-C15	-C17	-C18	31(1)
O14	-C15	-C17	-C18	-150(1)
O23	-C19	-C20	-O25	1(1)
O25	-C20	-C21	-O27	-2(1)

Molecules in the crystal are linked by hydrogen bonding through the chloride anion, which interacts equally with the protonated N2 atom and the carboxylic 013 atom (Table 4). Molecules are forming chains along the C axis by means of this hydrogen bonding. Figure 2 shows one of the four different chains which define the crystal packing.

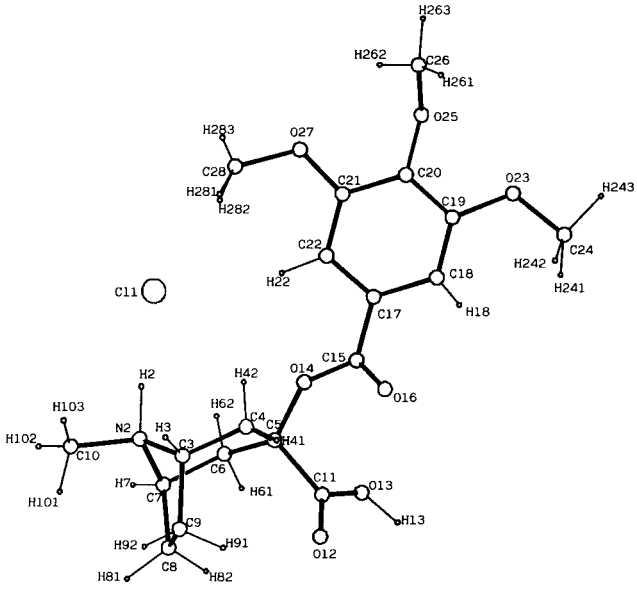


Figure 1. Pluto view of the molecule [8], showing the atom numbering.

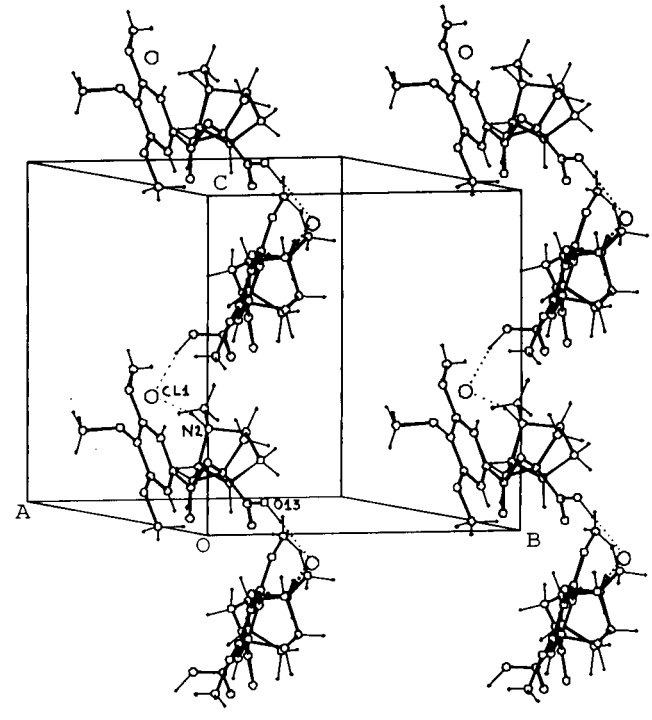


Figure 2. Packing in the unit cell with X range between 0.2 and 0.6.

Q_T = 0.665(7), ϕ_2 = 173(1) and θ_2 = 145(1). The five membered ring is in an envelope conformation with N atom 0.70(1) Å from the mean plane defined by the other four atoms.

The two substituents at the C5 atom deviate from the pseudo-mirror plane of the bicyclic system, both COO groups being in an approximate perpendicular orientation (angle between them: 104.4(7)°). On the other hand, the trimethoxyphenyl group is planar, except for the C26 atom in the *p*-position which is directed out from the mean plane defined by the aromatic ring; the COO group is twisted with respect to this plane, the angle between them being 148(4)°.

NMR Spectra.

The ^1H and ^{13}C nmr data of **4a-d** are summarized in Tables 5, 6 and 7; assignments of protons and carbons resonances were made from our studies of the corresponding hydroxyesters [1] and the data of several tropane derivatives studied by us [10-12]. In the case of the ^{13}C nmr assignments, signal multiplicity obtained from off-resonance decoupled spectra, were taken into consideration. In all cases, the coupling constants have practically the same values (Table 6) and $^3JH2(4)\beta$–H1(5) is greater than

Table 4

Hydrogen Contacts

N2-H2	H2...Cl1	N2...Cl1	N2-H2...Cl1
1.1(1)Å	2.0(1)Å	3.10(1)Å	166(6)°
O13-H13	H13...C11 [a]	O13...C11 [b]	O13-H13...C11 [a]
1.0(1)Å	2.0(1)Å	2.95(1)Å	157(7)°
0.13 [b] -H13 [b]	H13 [b]...C11	C13 [b]...C11	O13 [b]-H13 [b]..C11
1.0(1)Å	2.0(1)Å	2.95(1)Å	157(7)°

[a] X, 1/2-Y, -1/2+Z. [b] X, 1/2-Y, 1/2+Z.

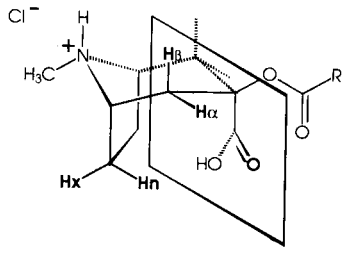


Figure 3. Preferred conformation in solution for compounds **4a-d**.

Table 5

^{1}H Chemical Shifts δ (ppm) for Compounds **4a-d** in Perdeuteriomethanol

Chemical shifts [a] δ (ppm)	**4a**	**4b**	**4c**	**4d**
H1(5) (brs)	3.94	4.02	3.88	3.83
W1/2	≈9.5Hz	≈9.6Hz	≈9.2Hz	≈9.2Hz
H2(4)α	2.96 (dd)	3.07 (d)	2.99 (dd)	2.87 (d)
H2(4)β (dd)	2.50	2.56	2.50	2.24
H6(7)x(m) [b]	2.22	2.33	2.25	2.16
H6(7)n(m) [b]	2.22	2.33	2.25	2.16
CH_3N(s)	2.73	2.82	2.75	2.65
H2'(6')	8.11 (m)	7.95 (m)	7.21 (s)	
H3'(5')	7.41 (m)	7.21 (m)		
Ar(m) [b]				7.19
CH(s)				5.0
CH_3OAr(s)			3.74	
$2CH_3OAr$(s)			2.79	

[a] Abbreviations: br, broad; d, doublet; dd, doublet of doublets; m, multiplet; s, singlet. δ values were deduced by first order analysis of the spectra; error ±0.05. [b] Multiplets of low resolution; tabulated chemical shifts correspond to the center of the multiplets.

Table 6

^{1}H-^{1}H Coupling Constants J (Hz) for Compounds **4a-d** in Perdeuteriomethanol

Coupling constants [a] J (Hz)	**4a**	**4b**	**4c**	**4d**
H1(5)-H2(4)α	2.2		3.2	
H1(5)-H2(4)β	4.1	4.1	4.0	4.0
H2(4)α-H2(4)β	-14.8	-15.3	-14.7	-14.3
H2'(6')-H3'(5')	8.6	8.9		
H2'(6')-F		5.5		
H3'(5')-F		8.9		

[a] Error ±0.2 Hz.

^{3}JH2(4)α-H1(5) and, consequently, the dihedral angle H2(4)α-C-C-H1(5) is greater than H2(4)β-C-C-H1(5) according to the Karplus relationship [13]. This is also more consistent with a chair flattened conformation than with a boat conformation for the piperidine ring since latter form should not only give a value of *ca.* 10 Hz for ^{3}JH2(4)β-H1(5) but also the signal corresponding to H1(5) should appear as an apparent doublet, a common feature in previously reported systems that adopt the boat conformation [14]. In the ^{13}C nmr spectra, the chair conformation adopted by the piperidine ring is confirmed by the C2(4) chemical shifts (Table 7). For a boat conformation these carbon signals would be shifted to higher field because of the steric compressing effect due to the eclipsing between H2(4)β and H1(5) hydrogen atoms [14].

Table 7

^{13}C Chemical Shifts δ (ppm) for Compounds **4a-d** in DMSO-d_6

	4a	**4b** [a]	**4c**	**4d**
C1(5)	62.01	64.35	60.65	61.28
C2(4)	38.22	39.61	39.08	37.58
C3	75.69	76.32	75.03	75.33
C6(7)	23.54	24.38	23.44	22.94
CH_3N	37.62	39.11	38.67	36.83
C=Oα	172.98	174.45	173.25	172.33
C=Oβ	164.56	165.89	164.81	170.91
C1'	128.18	127.01	124.43	138.22
C2'(6')	131.59	133.59	107.08	128.37
C3'(5')	129.36	116.76	152.98	128.31
C4'	139.20	167.57	142.28	127.00
CH				55.19
CH_3OAr			61.86	
$2CH_3OAr$			56.33	

[a] JC4' -F = 253.5 Hz; JC3' (5') -F = 22.2 Hz; JC2'(6')-F = 9.6 Hz and JC1'-F = 3.0 Hz.

We propose for compounds **4a-d** hydrochlorides in solution a preferred conformation similar to that observed for **4c** in the solid state. The relative spatial dispositions of the acyloxy and carboxylic groups are shown in Figure 3 and correspond to the more favourable arrangement in which the steric interactions with H2(4)β and H6(7)n protons respectively can be minimized. The O-CO-R residue lies practically in the symmetry plane of the bicyclic system, while the H2(4)α protons seem to be near to the oxygen atom and almost in the plane of the carboxylic moiety. The arylcarbonyloxy group is conjugated in **4a-c**. Moreover only one protonated *N*-epimer (of the two possible) arise as a result of the axial mode of proton uptake at the piperidine nitrogen atom.

These conclusions are supported by the following facts. The $\Delta\delta$H2(4)α-δH2(4)β ≈ 0.5 ppm can be partially attributed to the deshielding effect exerted by the CO group (COOH) on H2(4)α. Moreover, the spatial disposition assumed by the carboxylic group also explains the similar chemical shifts of H6(7)n and H6(7)x (see Table 5). The normal values found for δC2(4) (Table 7) also account for the situation of the OCOR residue far away of the H2(4)β protons. The δH and δC values for the arylcarbonyloxy

moieties account for conjugation between these groups in **4a-c**. For an axial position of the N-CH_3 group, a syn-diaxial γ-shielding effect on H2(4)β would shift δC2(4) to higher field at about 6 ppm [15].

With regard to δC1(5), δC2(4) and δC6(7) values of compounds **4a-c**, these are greatly similar with that reported for *N*-ethylnortropane-3-spiro-5'-oxazolidine-2',4'-dione hydrochloride (axial protonation) [15].

Biological Results.

The inhibitory ability of compounds **4** upon ^{3}H-GABA binding to synaptosomal brain membranes was assayed by using a GABA dose-response curve as a reference. All of these compounds were assayed at a range of increasing concentrations (from 3.10^{-8} *M* to 3.10^{-5} *M*). Only **4a** displayed some inhibitory effect upon ^{3}H-GABA binding, since it was able to inhibit it by about 35% of ^{3}H-GABA in a dose-response manner.

EXPERIMENTAL

All melting points were taken in open capillary tubes in a Electrothermal IA6304 apparatus, and are uncorrected. The elemental analyses were carried out in a Perkin-Elmer Elemental Analyzer model 240E. The ir spectra were recorded in the solid state (potassium bromide) using a Perkin-Elmer 883 spectrophotometer.

The ^{1}H nmr spectra were carried out using 4% w/v perdeuteriomethanol solutions. The spectra of compounds **4a** and **4c** were recorded at 400 MHz on a Brucker AM-400 spectrometer at a spectral width of 5000 Hz in 16 K memory and acquisition time of 1.638 s over 180 transients. The spectra of compounds **4b** and **4d** were recorded a 300 MHz using a Varian UNITY-300 spectrometer at a spectral width of 4000 Hz in 64 K memory and acquisition time of 3.0 s over 64 transients. In the latter cases resolution enhancement using LB = -0.80, GF = 0.50 and GFS = 0.20 was applied. All the proton resonances could be assigned. The H1(5) signal appears as a non-resolvable wide singlet. The H2(4)α and H2(4)β signals were assigned on the basis of the values of the respective couplings with H1(5) protons for related systems [10-12]. The H1, H2α and H2β protons (or H5, H4α and H4β) form a three-spin AMX system whose analysis leads to the establishment of their protonic parameters given in Tables 5 and 6. The aromatic protons of the Ar group appear as a four-spin AA'XX' system for Ar = *p*-Cl-C_6H_4 and for Ar = *p*-F-C_6H_4 were considered as parts of the five-spin AA'MM'X system formed by the aromatic protons and the fluorine atom. The analysis of the corresponding signals allowed the establishment of the chemical shifts (Table 5) and coupling constants ^{3}JH2'-H3' = ^{3}JH5'-H6', assuming that ^{5}JH2'-H5' = ^{5}JH3'-H6' = 0 Hz, as well as the respective JH-F (Table 6).

The ^{13}C nmr spectra were obtained at 75.429 MHz on a Varian UNITY-300 spectrometer at a spectral width of 16501 Hz in 64 K memory, acquisition time of 1 s and relaxation delay of 1 s, using *ca.* 20% w/v DMSO-d6 solutions. Two types of spectra were recorded: proton-noise decoupled spectra (to determine the chemical shifts) and off-resonance decoupled spectra (to help assign the signals).

All measurements were carried out at 298°K using TMS as the internal standard.

Synthesis and purification of tropinone (**1**) has been previously described [16,17].

3β-Hydroxy-*N*-methyl-8-azabicyclo[3.2.1]octane-3α-carbonitrile (**2**) [1].

To a stirred solution of the tropinone (10 g, 0.072 mole) in water (16 ml) and aqueous hydrochloric acid (12 ml, 6*N*), was added dropwise a solution of ammonium chloride (6.42 g, 0.12 mmole) in water (20 ml).

The mixture was externally cooled at 0° and potassium cyanide (4.68 g, 0.072 mole) in water (15 ml) was added very slowly (total addition time, ≈2 hours). The stirring was maintained during this operation, after 30 minutes the cyanohydrin was separated as a white solid. This was filtered and washed successively with different cold solvents: water (8 ml), ethanol (20 ml) and diethyl ether (20 ml). The product was keeping *in vacuo* over phosphorus pentoxide during at least 2 days after its use, and stored *in vacuo* to prevent its decomposition, yield 8.4 g (70%), mp 135-137°; ir (potassium bromide): ν OH, 3300-2400, ν CN, 2220 cm^{-1}.

3β-Hydroxy-*N*-methyl-8-azabicyclo[3.2.1]octane-3α-carboxylic Acid Hydrochloride (**3**).

The cyanohydrin **2** (0.996 g, 6 mmoles) was added portionwise to aqueous hydrochloric acid (15 ml, 12*M*) externally cooled at 0°. The mixture was magnetically stirred and maintained at 0° during this operation. The mixture was stirred at -2° for 30 minutes and kept at -5° for 24 hours. The solution was refluxed for 6 hours, allowed to cool at room temperature and the solvent evaporated under reduced pressure. The residue was treated with acetone and the title compound was separated as a white solid. This was filtered and dried *in vacuo* over phosphorus pentoxide during at least 1 day. After this time the product was purified by crystallization from ethanol, 1.19 g (90% yield) of the acid hydrochloride were obtained, mp 270-272° dec; ir (potassium bromide): ν OH, 3325, ν CO, 1728 cm^{-1}.

Anal. Calcd. for $C_9H_{15}NO_3$·HCl: C, 48.76; H, 7.25; N, 6.32. Found: C, 49.01; H, 7.42; N, 6.45.

Synthesis of Acyloxycarboxylic Acids Hydrochlorides **4a-d**. General Procedure.

The corresponding acid chloride (2.5 mmoles) in dry pyridine (5 ml) were added dropwise over a cooled (0°) and magnetically stirred solution of the carboxylic acid hydrochloride **3** (0.443 g, 2 mmoles) in dry pyridine (5 ml). The reaction mixture was magnetically stirred at room temperature for 10 days. The pyridine was removed *in vacuo* and the residue was treated with acetone to yield the corresponding acyloxy carboxylic acid hydrochloride as a white solid which was purified by crystallization from ethanol.

3β-(4'-Chlorobenzoyloxy)-*N*-methyl-8-azabicyclo[3.2.1]octane-3α-carboxylic Acid Hydrochloride (**4a**).

This compound was obtained in 56% yield, mp 181-184°; ir (potassium bromide): ν CO 1719 and 1782 cm^{-1}; pmr (see Tables 6 and 7); cmr (see Table 8).

Anal. Calcd. for $C_{16}H_{18}ClNO_4$·HCl: C, 53.35; H, 5.32; N, 3.89. Found: C, 53.40; H, 5.15; N, 4.14.

3β-(4'-Fluorobenzoyloxy)-*N*-methyl-8-azabicyclo[3.2.1]octane-3α-carboxylic Acid Hydrochloride (**4b**).

This compound was obtained in 60% yield, mp 216-218°; ir (potassium bromide): ν CO 1733 cm^{-1}; pmr (see Tables 6 and 7); cmr (see Table 8).

Anal. Calcd. for $C_{16}H_{18}FNO_4 \cdot HCl$: C, 55.90; H, 5.57; N, 4.07. Found: C, 55.63; H, 5.90; N, 4.41.

3β-(3',4',5'-Trimethoxybenzoyloxy)-*N*-methyl-8-azabicyclo[3.2.1]-octane-3α-carboxylic Acid Hydrochloride (**4c**).

This compound was obtained in 70% yield, mp 224-225°; ir (potassium bromide): ν CO, 1725 cm^{-1}; pmr (see Tables 6 and 7); cmr (see Table 8).

Anal. Calcd. for $C_{19}H_{25}NO_7 \cdot HCl$: C, 54.88; H, 6.30; N, 3.37. Found: C, 54.65; H, 6.21; N, 3.72.

3β-(Diphenylacetoxy)-*N*-methyl-8-azabicyclo[3.2.1]octane-3α-carboxylic Acid Hydrochloride (**4d**).

This compound was obtained in 48% yield, mp 210-211°; ir (potassium bromide): ν CO, 1731 cm^{-1}; pmr (see Tables 6 and 7); cmr (see Table 8).

Anal. Calcd. for $C_{23}H_{25}NO_4 \cdot HCl$: C, 66.42; H, 6.30; N, 3.37. Found: C, 66.31; H, 6.42; N, 3.56.

Biochemical Methods.

Membrane Preparations.

Crude synaptosomal brain membranes were prepared as described previously Zukin *et al.* [18]. Protein assays were carried out by the method of Bradford [19] using bovine serum albumin as a standard.

Binding Assay.

$GABA_B$ binding was performed in rat synaptic membranes essentially as described Hill and Bovery [20] with minor modifications. Briefly, 200 μg of membrane proteins were incubated with 10 n*M* ^{3}H-GABA, 40 μ*M* isoguvacine (as a $GABA_A$ receptor blocker) and increasing concentrations (10 n*M* - 3 μ*M*) unlabelled GABA in 50 m*M* Tris/2.5 m*M* of calcium chloride. The incubation was carried out at 25° for 10 minutes. The reaction was stopped by centrifugation at 14.00 g for 10 minutes. The pellet was disrupted in 1*N* sodium hydroxide and radioactivity measured. Specific $GABA_B$ binding was estimated as the difference between "total binding" and "non-specific binding" (binding in the presence of 100 μ*M* unlabelled GABA).

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