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Inhibitors of GABA Uptake. Syntheses and ^{1}H NMR Spectroscopic Investigations of Guvacine, (3*RS*,4*SR*)-4-Hydroxypiperidine-3-carboxylic Acid, and Related Compounds

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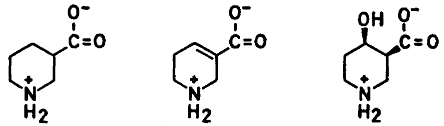
The syntheses of (3*RS*,4*SR*)-4-hydroxypiperidine-3-carboxylic acid (*8a*) and guvacine (1,2,5,6-tetrahydropyridine-3-carboxylic acid) hydrobromide (*9a*), both potent inhibitors of γ-aminobutyric acid (GABA) uptake, are described. Furthermore (3*RS*,4*SR*,5*SR*)- and (3*RS*,4*SR*,5*RS*)-4-hydroxy-5-methylpiperidine-3-carboxylic acids (*8c*) and (*8d*) and the guvacine analogues (*RS*)-5-methyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid (*10*) and 2,5,6,7-tetrahydro-1*H*-azepine-3-carboxylic acid and (1*R*,5*S*)-(−)-2-nortropene-2-carboxylic acid hydrobromides (*14*) and (*18*) have been synthesized. The compounds *8a,c,d*, *9a*, *10*, and *14* were prepared *via* catalytic hydrogenation of cyclic β-oxoesters and appropriate acid treatments of the intermediate β-hydroxy esters. Demethylation of ecgonine (*16*) followed by acid catalyzed hydrolysis and elimination reactions gave *18*. (*RS*)-Perhydroazepine-3-carboxylic acid and (1*R*,2*R*,5*R*)-(+)-nortropane-2-carboxylic acid hydrobromides (*15*) and (*19*) were obtained by catalytic hydrogenation of *14* and *18*, respectively. The relative stereochemistry of *6a,c,d*, *12*, *13*, and *19* was established by 270 MHz ^{1}H NMR spectroscopy. The relationship between structure and potency as inhibitors of GABA uptake of *8a,c,d*, *9a*, *10*, *14*, *15*, *18*, and *19* is discussed.



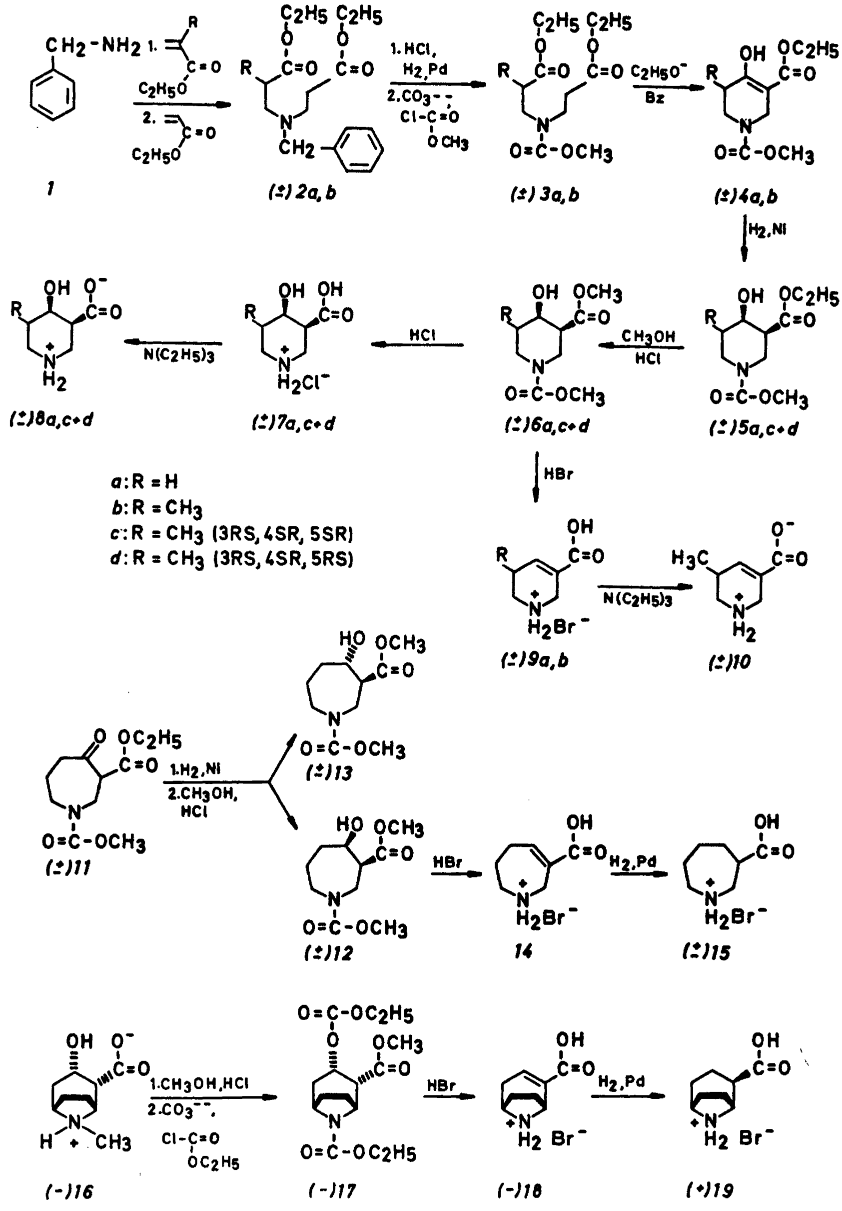
Nipecotic acid *Guvacine* *(±)8a*

Scheme 1.

Nipecotic acid (piperidine-3-carboxylic acid),[1-3] guvacine (1,2,5,6-tetrahydropyridine-3-carboxylic acid),[4] and (3*RS*,4*SR*)-4-hydroxypiperidine-3-carboxylic acid (*8a*)[5] (Scheme 1) are potent substrate-competitive inhibitors of the neuronal γ-aminobutyric acid (GABA) uptake process. The concentrations of (*R*)-(−)-nipecotic acid, guvacine, and compound *8a* required for 50 % inhibition of GABA uptake (IC_{50} values) are 5μM,[2] 8 μM,[4] and 12 μM,[5] respectively. These compounds seem to combine with the GABA transport carrier and penetrate the tissue.[2-5] Such compounds have pharmacological interest and may be useful tools for the study of the GABA transport carrier. However, molecular manipulations of these amino acids result in compounds with considerably reduced potency as inhibitors of GABA uptake, the IC_{50} values of *8c*, *8d*, *10*, and *15* being 260,[8] 349,[8] 547,[5] and 502 μM,[5] respectively. Compound *14*[5] and the conformationally rigid amino acids *18*[8] and *19*[5] are almost inactive. These findings demonstrate a pronounced substrate specificity of the GABA transport carrier.

This paper describes the syntheses of *8a*, its 5-methyl derivatives *8c* and *8d*, and the guvacine and nipecotic acid analoques *10*, *14*, *18*, *15*, and *19*. A convenient method for the preparation of guvacine hydrobromide (*9a*) has been developed. Guvacine hydrochloride has previously been synthesized on a very small scale.[7,8]

The β-oxoester *4b* was the only detectable product after Dieckmann cyclization of the unsymmetrical diester *3b* (Scheme 2). High pressure hydrogenation of *4a,b* gave the 3,4-*cis*-4-hydroxynipecotic acid derivatives *5a* and

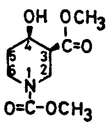


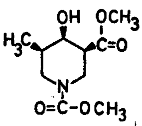
Scheme 2.

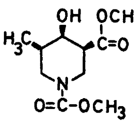
a separable mixture of *5c* and *5d*, respectively. The ethyl esters *5a,c,d* were transformed into the corresponding methyl esters in order to facilitate the analysis of the 270 MHz ^{1}H NMR spectra. Reduction of the β-oxoester *11*, however, gave a separable mixture of the *cis*- and *trans*-β-hydroxy esters *12* and *13* (Scheme 2).

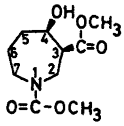
Appropriate treatmetnts of *6a,c,d* with hydrochloric acid gave the hydroxy amino acid chlorides *7a,c,d*, whereas the compounds *9a,b* were synthesized by prolonged treatments of *6a* and *6c* with hydrobromic acid. *N*-Demethylation of the methyl ester of ecgonine [(1*R*,2*R*, 3*S*,5*S*)-(−)-3-hydroxytropane-2-carboxylic acid]

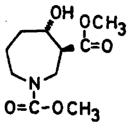
Table 1. Some chemical shifts (δ) and coupling constants (Hz) from the 270 MHz ^{1}H NMR spectra of *6a,c,d, 12, 13,* and *19.*[a]

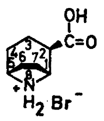

(±)6a


(±)6c


(±)6d


(±)12


(±)13


(+)19

	(±)*6a*	(±)*6c*	(±)*6d*	(±)*12*	(±)*13*	(+)*19*
δ_{1e}	—	—	—	—	—	4.15
δ_{2a}	3.43	3.19	*ca.* 3.7	3.38	3.17, 3.22	2.88
δ_{2e}	4.05	4.28	*ca.* 3.7	3.92, 4.07	3.79, 3.96	
δ_{3a}	2.64	2.58	2.81	2.71, 2.78	2.60, 2.66	
δ_{4a}	—	—	—	—	3.91	
δ_{4e}	4.32	4.17	3.79	4.32	—	
δ_{5a}	1.66	1.68	—	1.53	1.62	
δ_{5e}	1.84	—	1.98	2.06	1.90	3.93
δ_{6a}	3.30	2.82	3.23	1.69	1.62	
δ_{6e}	3.79	3.80	3.59	2.06	2.00	
δ_{7a}	—	—	—	3.20, 3.25	3.32	
δ_{7e}	—	—	—	3.64	3.56	
δC$-$COOCH_3	3.68	3.69	3.69	3.71, 3.72	3.69	
δN$-$COOCH_3	3.71	3.73	3.72	3.74, 3.75	3.75	
δC$-$CH_3	—	0.99	0.98	—	—	
J_{1e2a}	—	—	—	—	—	2.2
J_{2a2e}	-13.2	-13.0		-14.5	-14.8	
J_{2a3a}	10.7	12.0	10.0[b]	10.2	9.0	
J_{2e3a}	4.5	2.0	2.6[b]	3.0	3.75	
J_{3a4e}	2.5	4.4	3.4	3.0	—	
J_{3a4a}	—	—	—	—	9.0	
J_{4a5e}	—	—	—	—	2	
J_{4a5a}	—	—	—	—	9	
J_{4e5a}	3.0	*ca.* 4	—		—	
J_{4e5e}	4.4	—	5.3		—	
J_{5a5e}	-13.6	—	—			
J_{5a6a}	11.7	12.0				
J_{5a6e}	4.8	*ca.* 2				
J_{5e6a}	3.2	—	5.4			
J_{5e6e}	3.4	—	3.4			
J_{6a6e}	-13.4	-13.0	-13.6			
J_{7a7e}	—	—	—	-14		

[a] The spectra of *6a,c,d, 12,* and *13* were recorded in $CDCl_3$ and that of *19* in D_2O solutions. [b] Only the sum of J_{2a3a} and J_{2e3a} is precisely determined.

(*16*) by a modified von Braun procedure[9] followed by acid hydrolysis and dehydration of the intermediate *17* (Scheme 2) gave the rigid guvacine analogue *18*. Hydrogenation of *18* proceeded stereospecifically, *19* being the only detectable product.

The structure elucidation of the new products *2b*–*4b*, *5*–*10*, *12*–*15*, and *17*–*19* were based on elemental analyses, IR and ^{1}H NMR spectroscopy, in the cases of *9a,b*, *10*, *14*, and *18* supported by UV spectroscopy. In tetrachloromethane solution the β-oxoester *4b* (Scheme 2) exists in the enol-form as established by ^{1}H NMR spectroscopy. The relative configurations of *5*–*8*, *12*, *13*, and *19* were established by analysis of the 270 MHz ^{1}H NMR spectra of the methyl esters *6a,c,d*, *12*, and *13* and compound *19*, respectively.

In simple piperidine derivatives the equatorial proton on C(2) is found downfield from its axial counterpart.[10] The coupling constants between the C(3) proton and the two C(2) protons are typical for equatorial-axial and axial-axial configurations of these protons in *6a*. This is consistent with a predominantly equatorial orientation of the C(3) methoxycarbonyl group (Table 1). Furthermore the coupling constant for the C(3) and C(4) protons unequivocally indicates axial-equatorial orientation of the protons concerned and therefore a 3,4-*cis* configuration of *6a*. An analysis of the mutual coupling constants for the C(4) and C(5) protons supports this assignment. The vicinal coupling constants used in this assignment are in general agreement with those found in most 6-membered rings.[11] The geminal coupling constants found parallel data previously found in other piperidines.[12] The coupling patterns of the C(2), C(3), and C(4) protons in *6c* are very similar to those of the corresponding system in *6a* establishing equatorial and axial positions of the substituents at C(3) and C(4), respectively. The coupling constants for the C(5) proton indicate an equatorial orientation of the C(5) methyl group. These findings together are in agreement with the depicted relative configuration of *6c*. In *6d* the mutual coupling constants for the C(6) and C(5) protons indicate an axial orientation of the C(5) methyl group. A further analysis of the C(5), C(4), and C(3) proton coupling patterns reveals axial and equatorial orientations of the hydroxy and C(3) methoxycarbonyl groups, respectively.

In previous studies of piperidines[10] it has been suggested that the chemical shift difference between axial and equatorial protons on C(2) and C(6) ($\Delta\delta$) would indicate the extent to which the nitrogen lone pair influences the two protons. The differences $\Delta\delta(H_{2a},H_{2e})$ and $\Delta\delta(H_{6a},H_{6e})$ found in *6a* would be consistent with the dominating contribution from the equatorial-axial orientation of the C(3) and C(4) substituents of *6a*. The $\Delta\delta$ values found for *6c* are larger, indicating an increased fixation in an equatorial-axial-equatorial arrangement of the C(3), C(4), and C(5) substituents. This tendency is reflected in the values of the axial-axial coupling constants, where $J_{2a3a} = 12.0$ Hz would be consistent with an almost locked conformation. In *6d* the spectrum is quite different. The two protons at C(2) are almost coinciding in chemical shift at a value of *ca.* 3.7 ppm, the lack of accuracy arising from the near coincidence of the signal with those of the methoxy groups. The chemical shift difference between the two protons at C(2) can be judged from the triplet structure of the C(3) proton in the ABX pattern formed by the three protons at C(2) and C(3). The vanishingly small chemical shift difference on C(2) and the reduced chemical shift difference on C(6) as well as the smaller value of J_{2a3a} in *6d* (10.0 Hz) all point to a conformational equilibrium where equatorial-axial-axial orientations of the C(3), C(4), and C(5) substituents represent the preferred but not the exclusive conformation.

The 270 MHz ^{1}H NMR spectra of *12* and *13* were analysed as far as the complexity of the spectra permitted. Interpretation of the spectra, recorded at 293 K, was complicated by the fact that both molecules gave rise to two sets of signals due to hindered rotation of the urethane group. This is reflected in dual values for several chemical shifts while the coupling constants determined in both conformers were identical. The coupling constants clearly indicate that the arrangement of the substituents in position 3 and 4 are equatorial-axial in *12* and equatorial-equatorial in *13*. The observed values for chemical shifts and coupling constants are in general agreement with the data given for *6a*. In the spectrum of *19* a merging and very complex pattern of signals from the C(3), C(4), C(6), and C(7) protons is observed. It is not possible to establish the orientation of the carboxyl group of *19* on the basis of the coupling constant for the equatorial C(1) proton and the proton on C(2). However, the total width of the pattern originating in the C(2) proton reveals the existence of an axial-axial coupling between this proton and the axially oriented C(3) proton thus indicating an equatorial orientation of the carboxyl group.

EXPERIMENTAL

Unless otherwise stated, the determination of melting points and elemental analyses and the recording of IR, UV, and 60 MHz ^{1}H NMR spectra were accomplished as described in a previous paper.[13] ^{1}H NMR spectra of compounds dissolved in D_2O were recorded by using sodium 3-(trimethylsilyl)propanesulfonate as an internal standard. The 270 MHz ^{1}H NMR spectra were obtained on a Bruker HX 270 S instrument operating at 293 and 303 K. Fourier transform method was used to obtain the spectra with a spectral width of 1500 Hz using 32 K data points. The detection was quadrature detection. Homodecoupling was used to verify the interpretations of the spectra and provide starting parameters for the analyses of the spectra. Nitrogen decoupling was possible using a Bruker probehead equipped with an additional nitrogen decoupling coil. The frequency was derived from a Bruker synthesizer model BS 100 and fed to the amplifier of the decoupling channel through a band pass filter before entering the probe. The decoupling power was 5 watt. The decoupling frequency used was 19 506 582 Hz. The proton spectra were analyzed using the program SIMEQ and a Varian 620/i computer. Due to the large chemical shift differences at 270 MHz, it is permissible to subdivide the total spin system into several subsystems containing typically 3 to 6 nuclei. After the initial assignment the spectra were iterated to a best fit using the ITRCAL program on a Nicolet 1180 computer with 80 K memory. Optical rotations were measured on a Perkin-Elmer polarimeter model 141. Thin-layer chromatography (TLC) and column chromatography (CC) were accomplished by using silica gel F_{254} plates (Merck) and silica gel (Woelm 0.063 – 0.1 mm), respectively. Columns were developed by stepwise gradient elution. The pK_A values were determined as described in a previous paper.[14]

(RS)-Ethyl N-benzyl-N-(2-ethoxycarbonylethyl)-2-methyl-3-aminopropionate (2b). To a solution of *1* (107 g; 1 mol) in ethanol (200 ml) was added ethyl methacrylate (137 g; 1.2 mol). The mixture was refluxed for 24 h, the solvent removed *in vacuo*, and the residue distilled to give (*RS*)-ethyl *N*-benzyl-2-methyl-3-aminopropionate (149.9 g; 68 %), b.p. 131 – 133 °C/80 Pa. Anal. $C_{13}H_{19}NO_2$: C, H, N. IR (film): 3330 (w), 3030 (w), 2970 – 2820 (m), 1725 (s), 1495 (m), 1450(m) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 7.17 (5 H, s), 3.98 (2 H, q, J 7 Hz), 3.63 (2 H, s), 3.0 – 2.1 (3 H, m), 1.42 (1 H, s), 1.33 – 0.97 (6 H, m).

A solution of (*RS*)-ethyl *N*-benzyl-2-methyl-3-aminopropionate (142.8 g; 0.65 mol) and ethyl acrylate (83.9 g; 0.84 mol) in ethanol (150 ml) was refluxed for 4 days. The solvent was removed *in vacuo*, and distillation of the residue gave *2b* (164.8 g; 79 %), b.p. 165 – 167 °C/47 Pa. Anal. $C_{18}H_{27}NO_4$: C, H, N. IR (film): 3030 (w), 2980 (m), 2810 (m), 1730 (s), 1495 (w), 1455 (m) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 7.18 (5 H, s), 4.2 – 3.7 (4 H, dq), 3.52 (2 H, s), 2.9 – 2.0 (7 H, m), 1.4 – 0.9 (9 H, dt + m).

(RS)-Ethyl N-methoxycarbonyl-N-(2-ethoxycarbonylethyl)-2-methyl-3-aminopropionate (3b). A solution of *2b* (79.95 g; 250 mmol) and aqueous hydrochloric acid (62.5 ml; 4 M) in ethanol (200 ml) was hydrogenated (*ca.* 250 kPa) in a PARR hydrogenation apparatus by using a 10 % Pd-C catalyst (5.6 g). The reaction mixture was filtered and evaporated *in vacuo*. To an ice-cooled solution of the residue in water (100 ml) was added with stirring an iced solution of potassium carbonate (86.94 g; 630 mmol) in water (100 ml) followed by addition of methyl chloroformate (28.20 g; 300 mmol). Stirring was continued at 0 °C for 30 min and at 25 °C for 1 h. The mixture was extracted with ether (3 x 200 ml). The combined and dried (K_2CO_3) ether phases were evaporated *in vacuo* and the residue distilled to give *3b* (46.4 g; 64 %), b.p. 143 – 145 °C/53 Pa. Anal. $C_{13}H_{23}NO_6$: C, H, N. IR (film): 2980 (m), 1730 (s), 1705 (s), 1485 (m), 1445 (m), 1410 (m) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 4.02 (4 H, q, J 7 Hz), 3.59 (3 H, s) 3.5 – 3.1 (4 H, m), 3.0 – 2.0 (3 H, m), 1.4 – 0.8 (9 H, m).

(RS)-Ethyl 1-methoxycarbonyl-4-hydroxy-5-methyl-1,2,5,6-tetrahydropyridine-3-carboxylate (4b). To a suspension of sodium (1.84 g; 80 mmol) in benzene-xylene (10:1) (80 ml) was added ethanol (9.2 g; 200 mmol). When the sodium was dissolved a solution of *3b* (23.2 g; 80 mmol) in benzene (40 ml) was added with stirring. The reaction mixture was left for 3 days at 25 °C. Upon addition of hydrochloric acid (25 ml; 4 M) the organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to give crude *4b* (14.3 g). CC [silica gel: 400 g; eluents: benzene containing ethyl acetate (5 – 15 %)] followed by distillation gave *4b* (8.1 g; 42 %), b.p. 124 – 128 °C/40 Pa. Anal. $C_{11}H_{17}NO_5$: C, H, N. IR (film): 2980 (w), 2870 (w), 1710 (s), 1655 (m), 1620 (m), 1450 (m), 1410 (m) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 12.17 (1 H, s), 4.6 – 3.8 (4 H, m), 3.8 – 3.0 (5 H, m), 2.7 – 2.1 (1 H, m), 1.6 – 0.7 (6 H, m).

(3RS,4SR)-Ethyl 1-methoxycarbonyl-4-hydroxypiperidine-3-carboxylate (5a). A solution of *4a*[15] (55.0 g; 0.24 mol) in ethanol (500 ml) was hydrogenated (*ca.* 10 MPa) by using a Ra-Ni W-2 catalyst (9 g). The filtered and evaporated reaction product was distilled to give *5a* (50.0 g; 90 %), b.p. 145 – 149 °C/9 Pa, m.p. 51.5 – 53.5 °C. Anal. $C_{10}H_{17}NO_5$: C, H, N. IR (film): 3600 – 3200 (m), 2990 – 2850 (several bands, m), 1725 (s), 1705 (s) cm^{-1}.

(3RS,4SR)-Dimethyl 4-hydroxypiperidine-1,3-dicarboxylate (6a). A solution of *5a* (26 g; 0.11 mol) in a methanolic solution of hydrogen chloride (500 ml; 5 %) was refluxed for 18 h. The evaporated reaction mixture was distilled to give *6a* (21.0 g; 86 %), b.p. 143 – 146 °C/9 Pa, m.p. 68.5 – 70.5 °C. Anal. $C_9H_{15}NO_5$: C, H, N. IR (KBr): 3450 (s), 3000 – 2830 (several bands, m), 1720 (s), 1685 (s), 1670 (s) cm^{-1}.

(3RS,4SR)-3-Carboxy-4-hydroxypiperidinium chloride (7a). A solution of *6a* (4.0 g; 18.4 mmol) in hydrochloric acid (40 ml; 5 M) was refluxed for 150 min. The evaporated oily reaction product was crystallized from water-glacial acetic acid [35 ml; 2:5)] to give *7a* (1.6 g;

48 %), m.p. 170.0 – 172.0 °C. Anal. $C_6H_{12}ClNO_3$: C, H, Cl, N. IR (KBr): 3600 – 3350 (s), 3160 – 2740 (several bands, s), 2720 – 2400 (several bands, m), 1720 (s), 1600 (m) cm^{-1}. ^{1}H NMR (60 MHz, D_2O): δ 4.6 – 4.4 (1 H, m), 3.5 – 2.8 (5 H, m), 2.2 – 1.8 (2 H, m).

(3RS,4SR)-4-Hydroxypiperidine-3-carboxylic acid zwitterion (8a). To a solution of *7a* (1.82 g; 10 mmol) in water (5 ml) was added a solution of triethylamine (1.06 g; 10.5 mmol) in ethanol (5 ml). To the filtered solution was added *N,N*-dimethylformamide (4 ml). Upon standing at 25 °C for 7 days *8a* (890 mg; 61 %) was isolated, m.p. 253 – 255 °C (decomp.). Anal. $C_6H_{11}NO_3$: C, H, N. IR (KBr): 3210 (s), 3100 – 2200 (several bands, s), 1615 (s), 1550 (s), 1540 (s) cm^{-1}. pK_A values (H_2O, 22 °C): 3.42 ± 0.05; 10.02 ± 0.05.

(3RS,4SR,5SR)-Ethyl 1-methoxycarbonyl-4-hydroxy-5-methylpiperidine-3-carboxylate (5c) and (3RS,4SR,5RS)-ethyl 1-methoxycarbonyl-4-hydroxy-5-methylpiperidine-3-carboxylate (5d). A solution of *4b* (4.0 g; 16 mmol) in ethanol (130 ml) was hydrogenated (*ca.* 3 MPa) by using an Ra-Ni W-2 catalyst (3 g) for 23 h. The filtered and evaporated reaction mixture was shown by TLC [eluent: benzene-ethyl acetate (4:1)] to consist of two compounds ($R_F = 0.24$ and 0.17). CC [silica gel: 200 g; eluents: benzene containing ethyl acetate (15 – 25 %)] of the crude product and ball-tube distillation of the separated components at 133 Pa (oven temperature 180 °C) gave pure *5c* and *5d*. *5c* (1.45 g; 37 %) had m.p. 79.0 – 79.5 °C. Anal. $C_{11}H_{19}NO_5$: C, H, N. IR (KBr): 3470 (m), 2950 (m), 2900 (m), 1725 (s), 1685 (s), 1480 (s), 1455 (m), 1440 (m), 1415 (m) cm^{-1}. ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.4 – 3.9 (m) and 3.67 (s) (a total of 7 H), 3.4 – 2.0 (5 H, m), 1.9 – 0.8 (7 H, m). *5d* (1.12 g; 29 %) had m.p. 45 – 46 °C. Anal. $C_{11}H_{19}NO_5$: C, H, N. IR (film): 3470 (m), 2955 (m), 2925 (m), 1730 (s), 1700 (s), 1685 (s), 1480 (m), 1445 (m), 1415 (m) cm^{-1}. ^{1}H NMR (60 MHz, $CDCl_3$): δ 4.10 (q, J 7 Hz) and 3.9 – 2.2 (m) (a total of 12 H), 2.2 – 1.5 (1 H, m), 1.5 – 0.7 (6 H, m).

(3RS,4SR,5SR)-Dimethyl 4-hydroxy-5-methylpiperidine-1,3-dicarboxylate (6c). A solution of *5c* (531 mg; 2.2 mmol) in methanolic hydrogen chloride (10 ml; 5 %) was refluxed for 17 h. Ball-tube distillation of the evaporated reaction product at 133 Pa (oven temperature 180 °C) gave *6c* (472 mg; 93 %), m.p. 80.0 – 81.0 °C. The IR spectrum was almost identical with that of *5c*.

(3RS,4SR,5SR)-3-Carboxy-4-hydroxy-5-methylpiperidinium chloride (7c). A solution of *6c* (1.25 g; 5.4 mmol) in aqueous hydrochloric acid (50 ml; 5 M) was refluxed for 75 min. Evaporation of the reaction mixture *in vacuo* and recrystallization (water – acetic acid) of the residue gave *7c* (486 mg; 46 %), m.p. 232.5 – 233.5 °C. Anal. $C_7H_{14}ClNO_3$: C, H, Cl, N. IR (KBr): 3400 (m), 3145 (m), 2940 (m), 2800 (m), 1720 (s), 1605 (m), 1465 (m), 1450 (m), 1420 (m) cm^{-1}. ^{1}H NMR (60 MHz, D_2O): δ 4.23 (1 H, slightly broadened s), 3.6 – 2.5 (5 H, m), 2.3 – 1.6 (1 H, m), 1.00 (3 H, d, J 7 Hz).

(3RS,4SR,5SR)-4-Hydroxy-5-methylpiperidine-3-carboxylic acid zwitterion (8c). To a solution of *7c* (100 mg; 0.51 mmol) in water (1 ml) was added a solution of triethylamine (57 mg; 0.56 mmol) in ethanol (1 ml). The mixture was left at 4 °C for 17 h. Recrystallization (water – ethanol) of crude *8c* gave pure *8c* (46 mg; 51 %), m.p. 116.0 – 117.0 °C. Anal. $C_7H_{13}NO_3,H_2O$: C, H, N. IR (KBr): 3700 – 3100 (s), 3100 – 2400 (several bands, m), 1590 (s), 1470 (m), 1395 (s) cm^{-1}. pK_A values (H_2O, 23 °C): 3.39 ± 0.02; 10.13 ± 0.04.

(3RS,4SR,5RS)-Dimethyl 4-hydroxy-5-methylpiperidine-1,3-dicarboxylate (6d). *6d* was synthesized as described above for *6c* by using *5d* (471 mg; 1.9 mmol) as a starting material. Purification of the crude product by ball-tube distillation at 133 Pa (oven temperature 180 °C) gave *6d* (404 mg; 92 %). The IR spectrum was almost identical with that of *5d*.

(3RS,4SR,5RS)-3-Carboxy-4-hydroxy-5-methylpiperidinium chloride (7d). *7d* was synthesized as described above for *7c* by using *6d* (1.25 g; 5.4 mmol) as a starting material. Recrystallization (water – acetic acid) of the crude product gave *7d* (297 mg; 28 %), m.p. 211.5 – 212.0 °C. Anal. $C_7H_{14}ClNO_3$: C, H, Cl, N. IR (KBr): 3445 (m), 3110 (m), 3000 (m), 2845 (m), 1720 (s), 1585 (m), 1470 (m), 1435 (m) cm^{-1}. ^{1}H NMR (60 MHz, D_2O): δ 4.0 – 3.7 (1 H, m), 3.7 – 2.6 (5 H, m), 2.4 – 1.8 (1 H, m), 1.07 (3 H, d, J 7 Hz).

(3RS,4SR,5RS)-4-Hydroxy-5-methylpiperidine-3-carboxylic acid zwitterion (8d). *8d* was synthesized as described above for *8c* by using *7d* (100 mg; 0.51 mmol) as a starting material. Recrystallization (water-ethanol) of the crude product gave *8d* (28 mg; 31 %), m.p. 234.0 – 235.0 °C. Anal. $C_7H_{13}NO_3,H_2O$: C, H, N. IR (KBr): 3600 – 3350 (m), 3350 – 2800 (several bands, s – m), 1730 (m), 1600 (s), 1470 (m), 1410 (s) cm^{-1}. pK_A values (H_2O, 23 °C): 3.28 ± 0.03; 9.99 ± 0.06.

Guvacine hydrobromide (3-carboxy-1,2,5,6-tetrahydropyridinium bromide) (9a). A solution of *6a* (13.5 g; 62 mmol) in aqueous hydrobromic acid (60 ml; 48 %) was refluxed for 24 h. Upon cooling of the reaction mixture to 5 °C pure *9a* (10.4 g; 80 %) crystallized, m.p. 280 °C (decomp.). Anal. $C_6H_{10}BrNO_2$: C, H, Br, N. IR (KBr): 3175 (m), 2950 (s), 2820 (m), 2620 – 2400 (several bands, w), 1720 (s), 1660 (m), 1580 (m) cm^{-1}. UV [methanol (log ε)]: 212 (4.00) nm. ^{1}H NMR (60 MHz, D_2O): δ 7.3 – 7.0 (1 H, m), 3.9 – 3.7 (2 H, q), 3.5 – 3.1 (2 H, t), 2.7 – 2.3 (2 H, m). pK_A values (H_2O, 22 °C): 3.50 ± 0.06; 9.85 ± 0.06.

(RS)-3-Carboxy-5-methyl-1,2,5,6-tetrahydropyridinium bromide (9b). A solution of *6c* (517 mg; 1.99 mmol) in aqueous hydrobromic acid (1.5 ml; 48 %) was refluxed for 24 h. Upon cooling of the reaction mixture to 24 °C TLC-pure *9b* crystallized (162 mg; 37 %) [R_F:

0.22; eluent: 1-butanol – acetic acid – water (4:1:1)]. An analytical sample was recrystallized (water – acetic acid) to give *9b*, m.p. 253.5 – 254.5 °C. Anal. $C_7H_{12}BrNO_2$: C, H, Br, N. IR (KBr): 3550 – 3300 (m), 3250 – 2500 (several bands, s – m), 1710 (s), 1660 (m), 1605 (m), 1400 (m) cm^{-1}. UV [methanol (log ε)]: 214 (4.00) nm. ^{1}H NMR (60 MHz, D_2O): δ 7.2 – 6.9 (1 H, m), 3.9 – 3.7 (2 H, broadened s), 3.7 – 3.2 (1 H, m), 3.1 – 2.5 (2 H, m), 1.17 (3 H, d, *J* 7 Hz).

*(*RS*)-5-Methyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid zwitterion (*10*).* *10* was synthesized as described above for *8c* by using *9b* (100 mg; 0.56 mmol) and a solution of triethylamine (63 mg; 0.62 mmol) in ethanol (2 ml). Recrystallization (water – ethanol) of the crude product gave *10* (37 mg; 47 %), m.p. 249.0 – 249.5 °C. Anal. $C_7H_{11}NO_2$: C, H, N. IR (KBr): 3600 – 3250 (m), 3200 – 2000 (several bands, m), 1660 (s), 1630 (s), 1550 (s), 1470 (m) cm^{-1}. UV [methanol (log ε)]: 210 (3.88) nm. pK_A values (H_2O, 23 °C): 3.29 ± 0.03; 9.85 ± 0.03.

*(3*RS*,4*SR*)-Dimethyl 4-hydroxyperhydroazepine-1,3-dicarboxylate (*12*) and (3*RS*,4*RS*)-dimethyl 4-hydroxyperhydroazepine-1,3-dicarboxylate (*13*).* A mixture of *12* and *13* was prepared from *11*[16] (2.56 g; 10.5 mmol) in ethanol (15 ml) by using 1.5 g of catalyst as described above for *5c,d*, followed by reflux of a solution of the crude reduction product in methanolic hydrogen chloride (150 ml; 15 %) for 24 h. CC [silica gel: 180 g; eluents: benzene containing ethyl acetate (15 – 25 %)] of the evaporated reaction product followed by ball-tube distillation at 60 Pa (oven temperature 180 °C) of the isolated components gave *12* (1.68 g; 69 %), m.p. 55.5 – 57.5 °C. Anal. $C_{10}H_{17}NO_5$: C, H, N. IR (KBr): 3450 (m), 2950 (m), 2930 (m), 1735 (s), 1705 – 1675 (s), 1485 – 1440 (m), 1410 (m) cm^{-1}. 349 mg (14 %) of *13* was obtained. Anal. $C_{10}H_{17}NO_5$: C, H, N. IR (film): 3450 (m), 2950 (m), 2930 (m), 1735 (s), 1705 – 1680 (s), 1485 – 1440 (m), 1410 (m) cm^{-1}.

*3-Carboxy-2,5,6,7-tetrahydro-1*H*-azepinium bromide (*14*).* A solution of *12* (370 mg; 1.6 mmol) in hydrobromic acid (1 ml; 48 %) was refluxed for 24 h. Evaporation *in vacuo* and recrystallization (water – isopropanol) of the residue gave *14* (225 mg; 63 %), m.p. 224.5 – 225.5 °C. Anal. $C_7H_{12}BrNO_2$: C, H, Br, N. IR (KBr): 3430 (m), 3150 – 2700 (several bands, s – m), 1715 (s), 1650 (m), 1575 (m), 1460 (m), 1420 (m) cm^{-1}. UV [methanol (log ε)]: 210 (4.00) nm. ^{1}H NMR (60 MHz, D_2O): δ 7.55 (1 H, t, *J* 7 Hz), 4.12 (2 H, s), 3.6 – 3.3 (2 H, m), 2.8 – 2.3 (2 H, m), 2.2 – 1.6 (2 H, m). pK_A values (H_2O, 23 °C): 3.71 ± 0.05; 10.20 ± 0.03.

*(*RS*)-3-Carboxyperhydroazepinium bromide (*15*).* A solution of *14* (490 mg; 2.2 mmol) in aqueous ethanol (150 ml; 35 %) was hydrogenated (*ca.* 300 kPa) in a PARR hydrogenation apparatus by using a 10 % Pd – C catalyst (100 mg). Evaporation *in vacuo* and recrystallization of the residue (water – acetic acid) gave *15* (368 mg; 74 %), m.p. 145.0 – 146.5 °C. Anal. $C_7H_{14}BrNO_2$: C, H, Br, N. IR (KBr): 3700 – 3300 (m), 3250 – 2900 (s), 2860 (m), 2750 – 2400 (several bands, m – w), 1730 (s), 1580 (m) cm^{-1}. ^{1}H NMR (60 MHz, D_2O): δ 3.7 – 2.9 (5 H, m), 2.4 – 1.6 (6 H, m). pK_A values (H_2O, 23 °C): 3.33 ± 0.04; 10.58 ± 0.04.

*(1*R*,2*R*,3*S*,5*S*)-(–)-Methyl 3-ethoxycarbonyloxy-8-ethoxycarbonylnortropane-2-carboxylate (*17*).* A solution of ecgonine hydrate (*16*) (3.0 g; 14.8 mmol) in methanolic hydrogen chloride (200 ml; 5 %) was refluxed for 4 h. Upon evaporation *in vacuo* an aqueous solution (7 ml) of the residue, adjusted to pH 10 by sodium hydroxide, was extracted with chloroform (3 x 30 ml). The combined, dried (Na_2CO_3), and evaporated organic phases were submitted to ball-tube distillation at 130 Pa (oven temperature 150 °C) to give ecgonine methyl ester (2.5 g; 85 %) as a TLC-pure oil [R_F: 0.11; eluent: 1-butanol – acetic acid – water (4:1:1)]. A solution of ecgonine methyl ester (2.5 g; 12.6 mmol) and ethyl chloroformate (14.0 g; 130 mmol) in 1,2-dichloroethane (70 ml) was refluxed for 3 days. Evaporation *in vacuo* and CC [silica gel: 180 g; eluents: dichloromethane containing ethyl acetate (20 – 50 %) and methanol (3 %)] of the oily residue followed by ball-tube distillation at 100 Pa (oven temperature 170 °C) gave *17* (1.63 g; 39 %), m.p. 92.0 – 93.5 °C. Anal. $C_{15}H_{23}NO_7$: C, H, N. $[\alpha]_D^{25}$ – 48° (*c* 0.91, ethanol). IR (KBr): 3600 – 3100 (m), 2980 (m), 2890 (w), 1735 (s), 1710 (s), 1480 (m), 1435 (s) cm^{-1}. ^{1}H NMR (60 MHz, CCl_4): δ 5.2 – 4.7 (1 H, m), 4.7 – 4.3 (2 H, m), 4.3 – 3.8 (4 H, dq, each *J* 7 Hz), 3.66 (3 H, s), 3.1 – 2.8 (2 H, q), 2.7 – 2.2 (1 H, m), 2.0 – 1.6 (4 H, m), 1.5 – 1.2 (6 H, dt, each *J* 7 Hz).

*(1*R*,5*S*)-(–)-2-Nortropene-2-carboxylic acid hydrobromide (*18*).* A solution of *17* (825 mg; 2.5 mmol) in aqueous hydrobromic acid (6 ml; 48 %) was refluxed for 5 h. Evaporation *in vacuo* and recrystallization (2-propanol – ether) of the residue gave *18* (389 mg; 66 %), m.p. 278.5 – 280.5 °C. Anal. $C_8H_{12}BrNO_2$: C, H, Br, N. $[\alpha]_D^{25}$ – 61° (*c* 0.98, water). IR (KBr): 3080 (s), 2890 (s), 2730 – 2440 (several bands, m – w), 1690 (s), 1640 (m), 1600 (m) cm^{-1}. UV [methanol (log ε)]: 212 (3.95) nm. ^{1}H NMR (60 MHz, D_2O): δ 7.2 – 7.0 (1 H, t, *J* 4 Hz), 4.8 – 4.6 (1 H, m), 4.5 – 4.2 (1 H, m), 3.4 – 2.4 (2 H, m), 2.4 – 1.9 (4 H, m). pK_A values (H_2O, 23 °C): 3.33 ± 0.03; 10.23 ± 0.05.

*(1*R*,2*R*,5*R*)-(+)-Nortropane-2-carboxylic acid hydrobromide (*19*).* *19* was prepared as described above for *15* from *18* (250 mg; 1.1 mmol) by using 50 mg of the catalyst. 203 mg (81 %) of *19* was obtained, m.p. 237.5 – 238.5 °C. Anal. $C_8H_{14}BrNO_2$: C, H, Br, N. $[\alpha]_D^{25}$ + 12 ° (*c* 0.98, water). IR (KBr): 3600 – 3300 (m), 3120 (m), 3000 – 2830 (s), 2800 – 2400 (several bands, m – w), 1730 (s), 1610 (m) cm^{-1}.

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