SYNTHESIS AND PHARMACODYNAMIC INVESTIGATION OF NEW ISOGUVACINE ANALOGUES WITH BENZOQUINOLIZINE SKELETON

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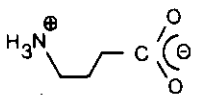
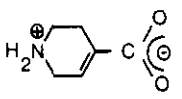
Abstract - Stereoisomer hydroxy-esters 15 - 19 as well as isoguvacine analogue 20 with benzo[a]quinolizine skeleton have been prepared via regioselective Dieckmann condensation of diester 9 followed by subsequent reduction and E_2-elimination. Compounds 16 and 20 have shown a considerable in vitro activity at the GABA/benzodiazepine receptor complex.

INTRODUCTION

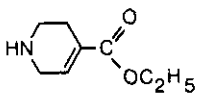
Extensive structure - activity relationship studies on conformationally restricted analogues of γ-aminobutyric acid (GABA) (1), the major inhibitory neurotransmitter in the brain[1-3], have led to the structure of isoguvacine (2) found potent and specific agonist at the bicuculline-sensitive $GABA_A$ receptor[4,5]. Existing in a zwitterionic form, isoguvacine poorly penetrates into the brain. To overcome this difficulty some non-ionic isoguvacine analogues with benzo[a]quinolizine ring-system (compounds 15 - 20) have been prepared and tested regarding to their activity at the $GABA_A$ receptor, the (-)-baclofen-sensitive $GABA_B$ receptor[6], and the benzodiazepine receptor[7] as well as in the GABA uptake system[8,9].



GABA (1)



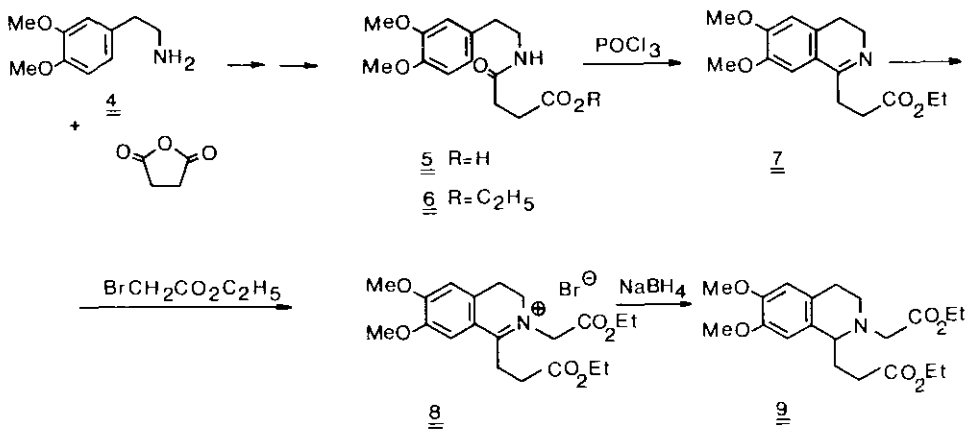
Isoguvacine (2)



(3)

CHEMISTRY

In order to synthesize benzo[a]quinolizines 15 - 20 homoveratrylamine (4) was acylated with succinic anhydride resulting in amide 5 which was immediately esterified to ester-amide 6. Bischler-Napieralski cyclization of 6 in acetonitrile with phosphorus oxytrichloride supplied 3,4-dihydroisoquinoline derivative 7 which upon treatment with ethyl bromoacetate in boiling methyl ethyl ketone gave iminium bromide 8 in crystalline form. The latter was reduced with sodium borohydride to give key-intermediate diester 9 in 40 % overall yield.



Dieckmann ring closure of diester 9 with potassium tert.-butoxide in boiling benzene yielded the enolizable β-keto-ester 10 as a sole product. Tlc investigation as well as the ^{1}H NMR spectrum of the crude product gave information on the extent of enolization which proved to be about 70 %. The enol form of 10 could be obtained in crystalline form from ethyl acetate and characterized by spectroscopic means. The appearance of two methylene carbons at δ 25.03 and 28.00 and the presence of merely one methylene signal belonging to a carbon atom neighbouring to the nitrogen at δ 45.52 in the ^{13}C NMR spectrum unambiguously proved the attendance of a $\Delta^{3,4}$ double bond, thus the C(4) position of the ethoxycarbonyl substituent in compound 10. Sodium borohydride reduction of 10 supplied four diastereomeric hydroxy-esters, 11, 12, 13 and 14, in a ratio of about 11 : 4 : 2 : 1, respectively. The depicted stereochemistry of the new compounds (11 - 14) were confirmed by ^{1}H and ^{13}C NMR spectroscopy (see Table 1 and Experimental).

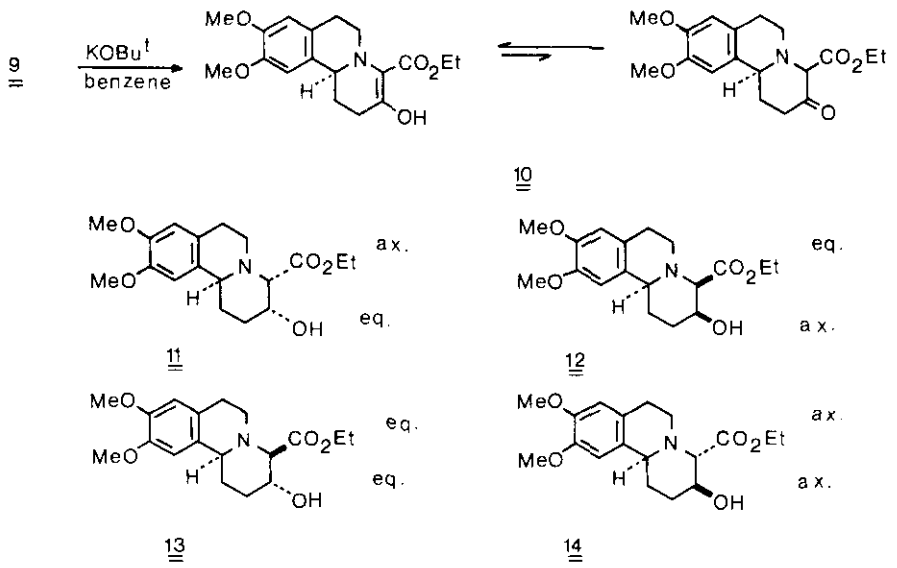


Table 1. ^{13}C NMR chemical shifts of compounds 10 - 14 in $CDCl_3$ solutions (δ values)

	10 enol form	11	12	13	14
C(1)	25.03	29.74	25.15	29.24	28.48
C(2)	28.00	30.91	31.38	33.30	27.38
C(3)	164.51	67.56	66.57	69.12	65.77
C(4)	114.48	68.59	70.90	74.90	69.61
C(6)	45.52	50.01	49.70	49.07	50.12
C(7)	29.68	29.46	29.20	29.36	29.90
C(7a)	126.22	126.56	127.00	126.66	126.70
C(8)	111.27	111.51	111.60	111.38	111.54
C(9)	147.75	147.40	147.49	147.74	147.47
C(10)	147.46	147.40	147.92	147.32	147.42
C(11)	110.01	108.93	108.75	108.69	108.73
C(11a)	129.88	129.71	129.30	129.24	130.20
C(11b)	56.49	54.72	62.63	61.73	54.90
OCH_3	55.85	55.86	55.94	55.88	55.87
OCH_3	56.04	56.02	56.19	56.08	56.03
C=O	170.48	171.92	171.16	172.51	170.67
$-CH_2-$	60.90	60.56	61.15	61.25	60.47
$-CH_3-$	14.20	14.48	14.19	14.30	14.36

Since the Dieckmann condensation which led to β-keto-ester 10 was carried out essentially under heterogeneous non-equilibrating conditions, the possibility of obtaining isomeric β-keto-ester 15 through the maintenance of homogeneity of the reaction was also studied. On performing the Dieckmann ring closure of diester 9 with sodium ethoxide in boiling ethanol, the exclusive formation of β-keto-ester 15 was observed. The ratio of keto and enol forms in the crude product proved to be about 1 : 4. The C(2) position of the ethoxycarbonyl substituent in 15 is supported by ^{13}C NMR data again; chemical shift values of methylene carbons vicinal to N(5) are δ 50.51 and δ 56.46, furthermore one benzylic as well as one allylic methylene carbon could be assigned at δ 28.86 and δ 29.99 for C(7) and C(1), respectively (see Table 2).

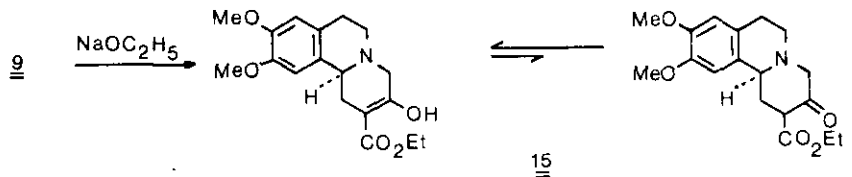
Table 2. ^{13}C NMR chemical shifts of compounds 15 - 20 in $CDCl_3$ solutions (δ values)

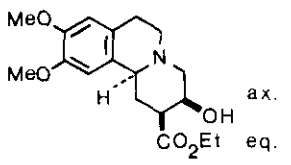
	15 enol form	16	17	18	19	20
C(1)	29.99	28.33	32.89	30.32	-	32.23
C(2)	96.34	46.74	50.14	43.32	39.94	129.28+
C(3)	168.18	65.68	67.55	67.18	63.66	136.26
C(4)	56.46	61.77	61.34	57.39	-	55.24
C(6)	50.51	52.02	51.90	51.77	-	50.92
C(7)	28.86	29.24	29.21	27.50	-	28.95
C(7a)	126.41	126.71	126.90	127.58	120.84*	126.48
C(8)	111.41	111.64	111.73	111.92	111.97	111.32
C(9)	147.52	147.59	147.45	147.64	148.61	147.51
C(10)	147.75	147.92	147.92	148.05	149.20	147.51
C(11)	108.92	108.52	108.80	108.75	108.12	108.58
C(11a)	128.85	128.69	128.54	126.90	124.18*	129.08+
C(11b)	58.82	61.63	61.76	58.09	57.17	58.27
OCH_3	55.84	55.92	55.91	55.99	55.97	55.85
OCH_3	56.22	56.20	56.26	56.24	56.35	56.10
C=O	171.86	172.84	174.03	173.94	173.0	166.60
$-CH_2-$	60.49	60.79	61.04	60.91	60.97	60.55
$-CH_3$	14.37	14.25	14.26	14.26	14.14	14.32

*,+ The values marked with identical symbols are interchangeable.

- Signals were not observable due to the small amount of material.

According to the results obtained by operating under two different sets of reaction conditions for the Dieckmann ring closure of diester 9, two different β-keto-esters (10 and 15) have been formed. Similar regioselectivity of Dieckmann-type condensation depending on reaction conditions has already been observed[10,11]. The differing regioselectivity can be explained by the formation of solid potassium enolate of 10 obtained from the kinetically controlled carbanion in one hand. On the other hand, in homogeneous media a total equilibrium between 10 and 15 affords the thermodynamically more stable quinolizidine-2-carboxylate derivative 15. This assumption is unequivocally supported experimentally when 10 was almost totally transformed into 15 in boiling ethanolic 2M $NaOC_2H_5$ solution in good accordance with Kline's previous observations[11].





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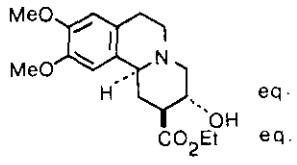

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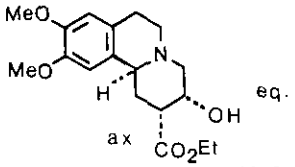

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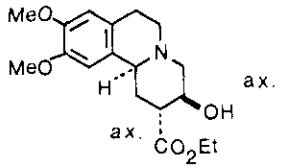

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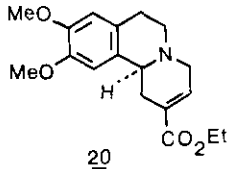

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Reduction of β-keto-ester 15, with sodium borohydride resulted in four stereoisomer hydroxy-esters, 16,17, 18 and 19, in a ratio of about 12 : 3 : 1 : 1, respectively. The Bohlmann bands[12] in the IR spectra of isomers 11 - 14 as well as 16 - 19, the typical chemical shift value of 11b-H in the ^{1}H NMR spectra,[13,14] furthermore the ^{13}C NMR measurement[15] (in particular the chemical shifts of C(6) and C(7) are indicative) proved the trans anellation of the B/C-rings. The relative steric position of the substituents either at C(3), C(4) or at C(2), C(3) were determined by ^{1}H and ^{13}C NMR spectroscopic means. It should be noted that an axial ethoxycarbonyl substituent at C(2) or at C(4) exerts deshielding effect on 11b-H (see Experimental), furthermore the γ-gauche effect of an axial substituent at C(2), C(3) or C(4) decreases the chemical shifts of the γ-carbon atoms compared with the corresponding equatorial substitution (see Tables 1,2.).

In order to obtain benzo[a]quinolizine derivative 20 possessing the isoguvacine moiety as a structural element of the molecule, water elimination was performed from hydroxy-ester 16 by treating it with thionyl chloride in pyridine. The easy water elimination of the major product 16 proves, at the same time, the relative steric position of substituents at C(2) and C(3) determined previously, which ensures optimal stereoelectronic conditions for E_2 elimination.

To increase the solubility in water, compounds 15 - 20 were transformed into their hydrochloride salt before pharmacodynamic testing.

RECEPTOR BINDING ASSAY

$GABA_A$ and benzodiazepine binding assays were performed as described earlier (see ref. 16 and 17), $GABA_B$ receptor and GABA uptake tests were made according to ref. 18. Neither of the compounds tested were found active at the $GABA_B$ receptor and GABA uptake system. Table 3 shows the IC_{50} values (concentration of drug causing 50 % inhibition of the binding of [^{3}H]GABA to the receptor) of the compound tested. Data were obtained from measurements in Tris - HCl buffer[16].

The esterification of isoguvacine (2) decreased its activity with one order of magnitude, and this value proved to be comparable with that of isoguvacine analogue 20 anellated with an isoquinoline carrier unit. Interestingly, compound 15 existing mostly in enol form and therefore being considered as a hydroxy substituted derivative of 20 proved to be almost inactive. Out of the four stereoisomer hydroxy-esters, 16 - 19, only 16 bearing equatorial substituent at C(2) and

Table 3. In vitro activity of isoquinoline derivatives 15 - 20 toward the GABA receptor.

Compound	[^{3}H]GABA specifically bound IC_{50} [μM]
1	0.008
2	0.010
3	0.20
15	100
16	3.9
17	91
18	56
19	44
20	0.22

an axial one at C(3) has shown appreciable activity toward the $GABA_A$ receptor. Table 4 shows the EC_{50} values (concentration of drugs causing 50 % enhancement of the binding of [^{3}H]flunitrazepam to the receptor) and the maximal enhancement at the benzodiazepine receptor brought about by compounds 1, 2 and 3. Such a stimulation of benzodiazepine binding is characteristic of GABA agonists[19,20]. Analogous behaviour is shown by compound 20. The GABA receptor activity of compound 20 was shifted toward lower affinity when the binding study was performed in physiological buffer, another indication of $GABA_A$ receptor agonist character[16,21].

Table 4. In vitro agonist potency of compound 20 as measured by the enhancement of [^{3}H]flunitrazepam binding.

Compound	[^{3}H]flunitrazepam specifically bound EC_{50} [μM]	max. enhancement [%]
1	1.0	150
2	3.5	82
3	35	61
20	-	66*

* measured at a concentration of 1 μM

After the above findings our intention is to turn toward the modification of the alkoxycarbonyl function of 20 in order to examine the structure - activity relationship and to increase the activity of this new type of GABA agonist agent possessing the benzo[a]quinolizine skeleton.

EXPERIMENTAL

Melting points are uncorrected. IR spectra were recorded on a Spectromom 2000 infrared spectrometer. ^{1}H NMR spectra were determined on Varian XL-100 as well as on Varian XL-400 instruments using deuteriochloroform as solvent and TMS as internal standard. Chemical shifts are reported as δ values. ^{13}C NMR spectra were determined on the same spectrometers operating in FT mode at 25 or 100 MHz, respectively. Mass spectra (MS) were obtained with an AEI MS-902 instrument (70 eV, direct insertion). Silica gel PF_{254} coated plates (E. Merck) were used for the purposes of qualitative tlc and preparative layer chromatography. Usual workup of the reaction mixtures was carried out by extraction of aqueous solutions or suspensions at pH 8.5-9 with dichloromethane. The combined organic layer was washed with water, dried with anhydrous $MgSO_4$, and finally the solvent was removed in vacuo. All reactions utilizing basic reagents were conducted under oxygen-free nitrogen.

Ethyl N-(3,4-Dimethoxyphenyl)ethyl-3-(aminocarbonyl)propionate (6)

To a stirred solution of homoveratrylamine (4) (20 g, 0.11 mol) in dry dichloromethane (400 ml) a solution of succinic anhydride (11 g, 0.11 mol) in dry dichloromethane 50 ml was added dropwise. After stirring 2 h the reaction mixture was evaporated in vacuo and the crude amide 5 was used for esterification without further purification. The solution of the crude amide 5 in dry ethanol (300 ml) in the presence of sulfuric acid (3 ml) was kept at room temperature for 48 h. After removing the solvent in vacuo the reaction mixture was dissolved in cold water (60 ml), basified with 5 % Na_2CO_3 solution and extracted with dichloromethane (3 x 50 ml). The combined organic layer was dried and evaporated. The remaining material was crystallized from ethyl acetate supplying ester amide 6 (26.5 g, 78 %), mp 106-108 ^{o}C; IR (KBr): 1720 (C=O), 1640 (N-C=O cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J=7Hz, 3H, CH_3), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.14 (q, J=7Hz, 2H, CH_2), 5.72 (broad s, 1H, NH), 6.75 (s, 2H) and 6.79 (s, 1H) aromatic protons; MS m/z (rel.int.): 309 (M^+, 5), 264 (7), 165 (100), 151 (18), 149 (5), 129 (4), 101 (11).

Ethyl 3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-1-yl)propionate (7)

Ester-amide 6 (8.0 g, 25.9 mmol) was dissolved in dry acetonitrile (200 ml) and phosphorus oxytrichloride (8 ml) was added. The reaction mixture was refluxed for 1 h, and then evaporated under reduced pressure. The residue was dissolved in cold water (100 ml), basified with Na_2CO_3 solution and extracted with chloroform (3 x 60 ml). The combined layer was dried and evaporated in vacuo to yield

amorphous 7 (4.9 g, 65 %). The crude product was treated with ethanol (50 ml) and 5M ethanolic hydrogen chloride (10 ml), then evaporated under reduced pressure and crystallized from ethanol-ether to give 7. HCl (3.9 g, 46 %), mp 126-129 °C; IR (KBr): 1730 (C=O), 1620 (C=N)cm^{-1}; ^{1}H NMR of liberated free base ($CDCl_3$) δ 1.25 (t, J=7Hz 3H, CH_3), 3.92 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.17 (q, J=7Hz, 2H, CH_2), 6.67 (s, 1H, ArH) and 7.08 (s, 1H, ArH); MS m/z (rel.int.): 291 (M^+, 4), 290 (16), 276 (100), 258 (14), 232 (5), 230 (8).

1-(Ethoxycarbonyl)ethyl-2-(ethoxycarbonyl)methyl-6,7-dimethoxy-3,4-dihydroisoquinolinium Bromide (8)

Following the above procedure *isoquinolinium salt 8 could be obtained when crude* 7 was dissolved in methyl ethyl ketone (100 ml) and ethyl bromoacetate (10 ml) and then refluxed for 8 h. The crystalline 8 (6.53 g, 55 % calculated for 3) was filtered off and washed with ether (2 x 15 ml), mp 173-175 °C; IR (KBr): 1740 (C=O), 1750 (C=O), 1600 (C=N)cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.23 (t, J=7Hz, 3H, CH_3), 1.36 (t, J=7Hz, 3H, CH_3), 2.90 (t, J=9Hz, 2H, CH_2), 3.33 (t, J=9Hz, 2H, CH_2), 3.68 (t, J=9Hz, 2H, CH_2), 3.95 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.13 (q, J=7Hz, 2H, CH_2), 4.32 (q, J=7Hz, 2H, CH_2), 5.42 (s, 2H, CH_2), 6.98 (s, 1H, ArH) and 7.50 (s, 1H, ArH); MS m/z (rel.int.): 377 (M^+-HBr, 18), 362 (4), 359 (5), 348 (14), 304 (100), 290 (12), 276 (10), 258 (35), 242 (4), 232 (5), 230 (8), 216 (4).

Ethyl 3-[2-(Ethoxycarbonyl)methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl]propionate (9)

To a solution of 8 (5.0 g, 10.9 mmol) in ethanol (100 ml) sodium borohydride was added in small portions at 0 °C and the reaction was monitored by tlc. The reaction mixture was neutralized with acetic acid and evaporated under reduced pressure. The residue was triturated with chloroform (100 ml), washed with water (2x25 ml), dried, and evaporated to give 9 (3.80 g, 92 %) as an oil. Crude 9 was transformed into its hydrochloride salt by treating it with 5N ethanolic hydrogen chloride to yield 9.HCl (3.81 g, 84 %), mp 150-152 °C; IR (KBr): 1740 (C=O), 1730 (C=O)cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, J=7Hz, 3H, CH_3), 1.25 (t, J=7Hz, 3H, CH_3), 3.40 (s, 2H, CH_2), 3.85 (s, 6H, 2xOCH_3), 4.12 (q, J=7Hz, 2H, CH_2), 4.18 (q , J=7Hz, 2H, CH_2), 6.56 (s, 1H) and 6.62 (s, 1H) aromatic protons; MS m/z (rel.int.): 379 (M^+, 0.5), 378 (0.5), 350 (0.5), 334 (7), 306 (6), 292 (4), 278 (100), 250 (7), 234 (2), 210 (2), 190 (3).

Ethyl (9,10-Dimethoxy-3-oxo-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-4-yl)-carboxylate (10)

To a solution of diester 9 (1.70 g, 4.5 mmol) in dry benzene (50 ml) potassium tert.-butoxide (0.60 g, 5.4 mmol) was added in one portion. The reaction mixture was refluxed for 4 h, then cooled to room temperature and neutralized with acetic acid. The solvent was removed in vacuo and the residue was suspended in dichloromethane (200 ml) and extracted with water (3 x 40 ml) at pH 8.5-9. The combined organic layer was dried and evaporated to yield amorphous 10 (1.0 g, 67 %). The enol form of 10 (0.69 g, 46 %) could be crystallized from ethyl acetate, mp 128-129 °C; IR (KBr): 1660 (C=O), 1580 (C=C)cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.34 (t, J=7Hz, 3H, CH_3), 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.25 + 4.34 (2 x q, J=7Hz, 2 x 1H, CH_2), 6.57 (s, 1H, ArH) and 6.60 (s, 1H, ArH), 11.47 (s, 1H, enol OH); MS m/z (rel.int.): 333 (M^+, 100), 316 (2), 304 (3), 287 (24), 286 (37), 260 (88), 259 (47), 258 (44), 244 (9), 232 (10), 231 (10), 230 (15), 218 (10), 205 (16), 203 (13), 191 (7), 190 (7), 189 (7), 188 (10).

Reduction of 10

To a stirred solution of β-keto-ester 10 (800 mg, 2.4 mmol) in dichloromethane (25 ml) and ethanol (25 ml) sodium borohydride was added in small portions. The reaction was monitored by tlc. After workup including purification by preparative tlc on silica gel PF_{254} (Merck) plates using dichloromethane-methanol (200:7 v/v) system 11 (362 mg, 45 %), 12 (145 mg, 18 %), 13 (64 mg, 8 %) and 14 (31 mg, 4 %) were obtained.

Ethyl (9,10-Dimethoxy-3α-hydroxy-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-4α-yl)carboxylate (11): mp 140-142 °C; IR (KBr): 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.29 (t, J=7Hz, 3H, CH_3), 3.81 (s, 6H, 2 x OCH_3), 3.83 (bd, J=4.5 Hz, 1H, 4-H), 3.95 (m, J=10 + 5 + 4.5 Hz, 1H, 3-H), 4.05 (dd, J=10 + 4Hz, 1H, 11b-H), 4.24 (q, J=7Hz, CH_2), 6.57 (s, 1H, ArH) and 6.65 (s, 1H, ArH); MS m/z (rel.int.): 335 (M^+, 26), 334 (19), 318 (5), 306 (15), 262 (100), 246 (17), 244 (5), 234 (9), 232 (3), 218 (43), 205 (7), 204 (6), 191 (26), 176 (10).

Ethyl (9,10-Dimethoxy-3β-hydroxy-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-4β-yl)carboxylate (12): mp 117-119 °C; IR (KBr): 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (t, J=7Hz, 3H, CH_3), 3.30 (bd, J=2.5 Hz, 1H, 4-H), 3.38 (dd, J=9+4.5 Hz, 1H, 11b-H), 3.81 (s, 6H, 2 x OCH_3), 4.10 (m, J=2.5+2.5+2.5 Hz, 1H, 3-H), 4.26 (q, J=7Hz, 2H, CH_2), 6.58 (s, 1H, ArH) and 6.70 (s, 1H, ArH); MS m/z (rel.int.): 335 (M^+, 21), 334 (19), 318 (4), 317 (7), 316 (7), 306 (13), 288 (3), 262 (100),

246 (3), 244 (6), 234 (8), 218 (31), 205 (4), 204 (3), 191 (17), 176 (6).

Ethyl (9,10-Dimethoxy-3α-hydroxy-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-4β-yl)carboxylate (13): mp 111-114 °C; IR (KBr): 1720 (C=O)cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (t, J=7Hz, 3H, CH_3), 3.30 (dd, J=10+4Hz, 1H, 11-bH), 3.80 (bd, J=7Hz, 1H, 4-H), 3.81 (s, 6H, 2 x OCH_3), 3.98 (m, J=9.5 + 7 + 4 Hz, 1H; 3-H), 4.30 (q, J=7Hz, 2H, CH_2), 6.56 (s, 1H, ArH) and 6.66 (s, 1H, ArH); MS m/z (rel.int.): 335 (M^+,19), 334 (17), 318 (4), 306 (13), 262 (100), 246 (5), 244 (6), 242 (7), 240 (9), 234 (6), 218 (19), 205 (4), 204 (3), 191 (13), 176 (6).

Ethyl (9,10-Dimethoxy-3β-hydroxy-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-4α-yl)carboxylate (14): amorphous, IR (KBr): 1720 (C=O)cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.30 (t, J=7Hz, 3H, CH_3), 3.64 (d, J=3Hz, 1H, 4-H), 3.82 (s, 6H, 2 x OCH_3), 3.92 (dd, J=10 + 4Hz, 1H, 11b-H) 4.21 (m, J = 3 + 3 + 3 Hz, 1H, 3-H), 4.21 (q, J=7Hz, 2H, CH_2), 6.58 (s, 1H, ArH) and 6.64 (s, 1H, ArH); MS m/z (rel.int.): 335 (M^+,17), 334 (14), 318 (2), 306 (22), 262 (100), 246 (3), 244 (3), 234 (8), 232 (7), 218 (17), 205 (8), 204 (3), 191 (25), 176 (7).

Ethyl (9,10-Dimethoxy-3-oxo-1,2,4,6,7,11bα)-hexahydro-3H-benzo[a]quinolizin-2-yl)-carboxylate (15). To a solution of diester 9 (1.70 g, 4.5 mmol) in dry ethanol (50 ml) freshly prepared sodium ethoxide (0.68 g, 10 mmol) was added and refluxed overnight. The reaction mixture was cooled to room temperature and neutralized with acetic acid, finally the solvent was removed in vacuo. The residue was suspended in dichloromethane (200 ml) and extracted with water (3 x 40 ml) at pH 8.5-9. The combined organic layer was dried and evaporated to give amorphous 15 (0.89 g, 59 %). The enol form of 15 (0.60 g, 40 %) could be obtained in crystalline form from ethyl acetate: mp 122-124 °C; IR (KBr): 1690 (C=O), 1550 (C=C)cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (t, J=7Hz, 3H, CH_3), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.28 (q, J=7Hz, 2H, CH_2), 6.61 (s, 1H, ArH) and 6.72 (s, 1H, ArH), 11.94 (bs, 1H, enol OH); MS m/z (rel.int.): 33.3 (M^+, 46), 332 (5), 304 (3), 288 (4), 287 (4), 286 (6), 260 (7), 259 (3), 258 (3), 205 (29), 192 (22), 191 (100), 190 (14), 176 (17).

Reduction of 15

To a stirred solution of β-keto-ester 15 (600 mg, 1.8 mmol) in dichloromethane (20 ml) and ethanol (20 ml) sodium borohydride was added in small portions. The reaction was monitored by tlc. After workup including separation by preparative tlc on silica gel PF_{254} (Merck) plates with dichloromethane-methanol (200:10 v/v) system, 16 (368 mg, 61 %), 17 (91 mg, 15 %), 18 (36 mg, 6 %) and 19 (25 mg, 4 %)

were obtained.

Ethyl (9,10-Dimethoxy-3β-hydroxy-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-2β-yl)carboxylate (16): mp 121-123 °C; IR (KBr): 1720 (C=O)cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.28 (t, J=7Hz, 3H, CH_3), 3.21 (dd, J=10.5 + 3 Hz, 1H, 11b-H), 4.22 (q, J=7Hz, 2H, CH_2), 4.30 (m, J=2.5 + 2.5 + 2.5 Hz, 1H, 3-H), 6.60 (s, 1H, ArH) and 6.72 (s, 1H, ArH); MS m/z (rel.int.): 335 (M^+, 37), 334 (44), 320 (14), 318 (2), 306 (19), 291 (14), 290 (23), 262 (23), 235 (11), 234 (23), 205 (21), 191 (100), 176 (11).

Ethyl (9,10-Dimethoxy-3α-hydroxy-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-2β-yl)carboxylate (17): mp 124-126 °C; IR (KBr): 1720 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.29 (t, J=7Hz, 3H, CH_3), 3.21 (dd, J=11 + 5 Hz, 1H, 11b-H), 3.82 (s, 6H, 2 x OCH_3), 4.08 (m, J=10 + 9.5 + 4.5 Hz, 1H, 3-H), 4.24 (q, J=7Hz, 2H, CH_2), 6.59 (s, 1H, ArH) and 6.69 (s, 1H, ArH); MS m/z (rel.int.): 335 (M^+, 44), 334 (52), 320 (6), 306 (26), 291 (11), 290 (20), 262 (20), 235 (9), 234 (21), 205 (19), 191 (100), 176 (10).

Ethyl (9,10-Dimethoxy-3α-hydroxy-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-2α-yl)carboxylate (18): amorphous, IR (KBr): 1720 (C=O)cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.32 (t, J=7Hz, 3H, CH_3), 3.59 (dd, J=10 + 3Hz, 1H, 11b-H), 3.85 (s, 6H, 2 x OCH_3), 4.10 (m, J = 9 + 8.5 + 4 Hz, 1H, 3-H), 4.28 (q, J=7Hz, 2H, CH_2), 6.61 (s, 1H, ArH) and 6.70 (s, 1H, ArH); MS m/z (rel.int.): 335 (48), 334 (48), 320 (6), 318 (3), 306 (29), 291 (23), 290 (33), 262 (25), 235 (13), 234 (28), 205 (23), 191 (100), 176 (11).

Ethyl (9,10-Dimethoxy-3β-hydroxy-1,2,4,6,7,11bα-hexahydro-3H-benzo[a]quinolizin-2α-yl)carboxylate (19): amorphous, ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J=7Hz, 3H, CH_3), 3.83 (s, 6H, 2 x OCH_3), 4.20 (q, J=7Hz, 2H, CH_2), 6.70 (s, 2H, aromatic protons); MS m/z (rel.int.): 335 (M^+, 43), 334 (43), 320 (7), 318 (3), 306 (28), 291 (21), 290 (32), 262 (25), 235 (13), 234 (25), 205 (21), 191 (100), 176 (13).

Ethyl (9,10-Dimethoxy-1,5,6,11bα-tetrahydro-4H-benzo[a]quinolizin-2-yl)carboxylate (20): Hydroxy-ester (16) was dissolved in dry pyridine (4 ml) and thionyl chloride was added. The reaction mixture was kept at room temperature overnight. Workup including preparative tlc on silica gel PF_{254} (Merck) plates by dichloromethane-methanol (200:10 v/v system) supplied amorphous 20 (45 mg, 24 %) which was crystallized as its hydrochloride salt: mp 157-159 °C; IR(KBr): 1700 (C=O), 1590 (C=C)cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J=7Hz, 3H, CH_3), 3.86 (s, 6H, 2 x OCH_3), 4.25 (q, J=7Hz, 2H, CH_2), 6.61 (s, 1H, ArH) and 6.74 (s, 1H, ArH), 6.96 (m,

J=4 + 2 + 2 Hz, 1H, 3-H); MS m/z (rel.int.): 317 (M^+, 43), 316 (15), 302 (1), 288 (2), 286 (1), 272 (5), 244 (7), 242 (2), 228 (2), 218 (2), 203 (2), 192 (14), 191 (100), 176 (22).

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