the resulting precipitate was filtered, washed with cold water, and dried in vacuo: yield 1.21 g (89%); mp 135–138 °C. Recrystallization from ethanol–water gave an analytical sample: mp 129–131 °C; IR (Nujol) 3300 (NH), 1710 (amide C=O), 1675 (amide C=O), 1650, 1540 (amide II) cm^{-1}; NMR ($CDCl_3$) δ 8.13 (s, 1, H-5), 7.91 (s, 1, H-5′), 7.86 (br s, 1, $NHCH_2CH_2$), 7.56 (br t, 1, $NHCH_2CH_2CH_2$), 3.88 (q, 2, $NHCH_2CH_2$), 3.59 (q, 2, $NHCH_2CH_2CH_2$), 3.30 (t, 2, $NHCH_2CH_2$), 2.61 (t, 2, $NHCH_2CH_2CH_2$), 2.13 (s, 3, CH_3S), 1.96 (p, 2, $CH_2CH_2CH_2$). Anal. ($C_{15}H_{17}F_3N_4O_2S_3$) C, H, N.

3-[2′-(2-Aminoethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfide Hydrochloride (13). To a solution of **12** (1.0 g, 2.3 mmol) in 25 mL of methanol was added 25 mL of concentrated ammonium hydroxide and the solution was allowed to stand for 18 h at room temperature. Removal of solvent in vacuo gave a residue which was extracted with ether (2 × 25 mL) and then taken to dryness. The residue (1 g) was recrystallized from ethanolic HCl to give an analytical sample (0.7 g, 85%): mp 209–210 °C; IR (Nujol) 3460 ($-NH_3^+$), 3315 (amide NH), 1640 (amide C=O), 1540 (amide II) cm^{-1}; NMR (D_2O) δ 8.14 (s, 1, H-5), 8.09 (s, 1, H-5′), 3.50 (m, 6, $NHCH_2CH_2$, $NHCH_2CH_2CH_2$), 2.63 (t, 2, $NHCH_2CH_2CH_2$), 2.13 (s, 3, CH_3S), 1.94 (p, 2, $CH_2CH_2CH_2$). Anal. ($C_{13}H_{19}ClN_4OS_3$) C, H, N.

3-[2′-(2-Acetamidoethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfide (14). Method A. A solution of **13** (100 mg, 0.26 mmol) in 3 mL of acetic anhydride containing 0.5 mL of pyridine was stirred at room temperature for 18 h. The solvent was removed in vacuo and the residue was washed with 5 mL of water. The crude product was dried in vacuo and the residue was washed with 5 mL of water. The crude product was dried in vacuo, giving 93 mg (91%) of **14**, mp 132–133 °C. Recrystallization from ethanol gave an analytical sample: mp 135–137 °C; IR (Nujol) 3340, 3280 (NH), 1675, 1640 (amide C=O), 1545 (amide II) cm^{-1}; NMR ($CDCl_3$) δ 8.10 (s, 1, H-5), 7.87 (s, 1, H-5′), 7.53 (br s, 1, $NHCH_2CH_2CH_2$), 6.23 (br s, 1, $NHCH_2CH_2$), 3.75 (q, 2, $NHCH_2CH_2$), 3.59 (q, 2, $NHCH_2CH_2CH_2$), 3.25 (t, 2, $NHCH_2CH_2$), 2.61 (t, 2, $CH_2CH_2CH_2$), 2.13 (s, 3, CH_3S), 2.00 (s, 3, CH_3CO), 1.96 (m, 2, $CH_2CH_2CH_2$). Anal. ($C_{15}H_{20}N_4S_3O_2$) C, H, N.

Method B. A solution of **8b** (1.50 g, 4.82 mmol) in 4 mL of 3-(methylthio)propylamine was heated at 80 °C for 16 h. The reaction mixture was poured into 50 mL of water, and the resultant solid was filtered and dried in vacuo to give 1.72 g (93%) of crude **14**, mp 133–135 °C. Recrystallization from ethanol gave an analytical sample identical in all respects with material prepared by method A.

[3-[2′-(2-Acetamidoethyl)-2,4′-bithiazole-4-carboxamido]propyl]dimethylsulfonium Chloride (1). A solution of **14** (260 mg, 0.68 mmol) in 2 mL of methyl iodide containing 0.5 mL of methanol was heated in a sealed tube at 70 °C for 20 h. Evaporation of the solvent gave the iodide salt as a hygroscopic yellow solid. This was converted to the chloride salt by passage of an aqueous solution through a column (1 × 5 cm) of Dowex 1X8 (chloride form). Lyophilization of the eluate gave 290 mg (98%) of **1** in analytical pure form: NMR (D_2O) δ 7.94 (s, 1, H-5), 7.78 (s, 1, H-5′), 3.46 (t, 2, NCH_2CH_2), 3.41 (t, 2, $NCH_2CH_2CH_2$), 3.33 (t, 2, NCH_2CH_2), 3.04 (t, 2, CH_2S), 2.88 [s, 6, $S^+(CH_3)_2$], 2.09 (p, 2, $CH_2CH_2CH_2$), 1.88 (s, 3, CH_3CO). Anal. ($C_{16}H_{23}ClN_4O_2S_3 \cdot 2H_2O$) C, H, N.

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Esters of Isoguvacine as Potential Prodrugs

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The syntheses of the methyl ester, butyl ester, (ethoxycarbonyl)methyl ester, and 11 (acyloxy)methyl esters of the potent γ-aminobutyric acid agonist isoguvacine (1,2,3,6-tetrahydropyridine-4-carboxylic acid) are described. The chemical stability of the esters and their in vitro rates of hydrolysis under approximately physiological conditions by nonspecific esterases from human serum were examined. A selected number of the esters were tested for antagonism of convulsions induced by bicuculline, isoniazide, and by electroschock. While the compounds showed only weak activities in the bicuculline and isoniazide tests, a good correlation between in vitro rates of enzymatic hydrolysis and the time of onset of the antagonism of the electroshock-induced convulsions could be found.

Decreased functions of the central γAbu (γ-aminobutyric acid) neutrotransmitter system contribute to the pathogenesis of certain psychiatric and neurological disorders.[1,2] Consequently, γAbu stimulation or replacement therapies may be relevant for the treatment of these diseases,[3] the γAbu-metabolizing enzyme (γAbu-T),[4] the γAbu uptake systems,[5,6] and, in particular, the γAbu receptors[7] being of primary interest as pharmacological sites of attack.

A variety of γAbu analogues with γAbu agonist activity have been developed.[8,9] Among these compounds, the potent γAbu agonists THIP (4,5,6,7-tetrahydroisoxazolo-

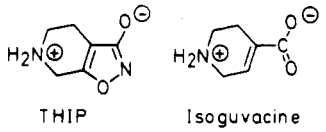

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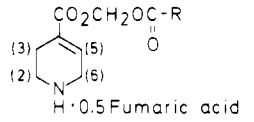
Table I. (Acyloxy)methyl Esters of Isoguvacine



no.	R	yield,[a] %	mp,[b] °C	formula[c]	NMR data, δ, for R[d]
5a	CH_3	14	150-151	$C_{11}H_{15}NO_6$	2.2 (3 H, s)
5b	CH_3CH_2	17	144-145	$C_{12}H_{17}NO_6$	1.05 (3 H, t), 2.50 (2 H, q)
5c	$CH_3(CH_2)_2$	13	138-139	$C_{13}H_{19}NO_6$	0.95 (3 H, t), 1.55 (2 H, m), 2.4 (2 H, t)
5d	$(CH_3)_2CH$	15	148-149	$C_{13}H_{19}NO_6$	1.1 (6 H, d), 2.6 (1 H, m)
5e	$CH_3(CH_2)_3$	35	146-148	$C_{14}H_{21}NO_6$	0.95 (3 H, t), 1.1-1.8 (4 H, m), 2.4 (2 H, t)
5f	$(CH_3)_2CHCH_2$	18	143-144	$C_{14}H_{21}NO_6$	0.95 (6 H, d), 2.0 (1 H, m), 2.35 (2 H, d)
5g	$(CH_3)_3C$	19	157-158	$C_{14}H_{21}NO_6$	1.20 (9 H, s)
5h	$CH_3(CH_2)_4$	25	147-148	$C_{15}H_{23}NO_6$	0.75-2.0 (9 H, m), 2.45 (2 H, t)
5i	$(CH_3CH_2)_2CH$	21	130-131[e]	$C_{15}H_{23}NO_6$	0.90 (6 H, t), 1.55 (4 H, m), 2.35 (1 H, m)
5j	C_6H_5	14	148-150[e]	$C_{16}H_{17}NO_6$	7.7-8.3 (5 H, m)
5k	C_6H_{11}[f]	11	150-151	$C_{16}H_{23}NO_6$	0.8-2.0 (11 H, m)

[a] The yields listed are those obtained from the sequence of reactions from compound 2 to the esters 5 (see Scheme I). [b] All of the compounds decomposed at the melting point. [c] All of the compounds were analyzed for C, H, N. [d] In D_2O. The values for the isoguvacine ring protons for all of the compounds, except 5j were as follows: for the protons at C(2), δ 3.45 (2 H, t); at C(3), 2.65-2.70 (2 H, m); at C(5), 7.10-7.15 (1 H, m); at C(6), 4.0 (2 H, m); and for the protons of the methylenedioxy group, 5.95-6.0 (2 H, s). The signals from the olefinic protons in fumaric acid were found at δ 6.55-6.65 (1 H, s). The data for compound 5j were as follows: for protons at C(2), δ 3.60; at C(3), 2.85; at C(5), 7.30; at C(6), 4.15; methylenedioxy group protons, 6.30; fumaric acid protons, 6.80. [e] Crystallized in some cases with 1 mol of fumaric acid. The hydrogen fumarate of 5i, mp 141-142 °C dec. Anal. ($C_{17}H_{25}NO_8$) C, H, N. The hydrogen fumarate of 5j, mp 148-149 °C dec. Anal. ($C_{18}H_{19}NO_8$) C, H, N. [f] Cyclohexyl.

[5,4-c]pyridin-3-ol) and isoguvacine (1,2,3,6-tetrahydropyridine-4-carboxylic acid), which interact specifically with the γAbu receptors,[10,11] have considerable pharmacological interest.

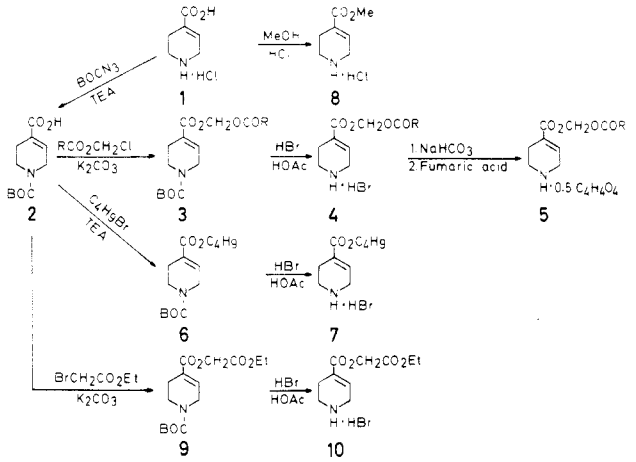
THIP and isoguvacine are structurally related zwitterionic compounds, but THIP is centrally active after systemic administration in animals, in agreement with the finding that THIP, in contrast to isoguvacine, is capable of penetrating the blood–brain barrier.[12] Isoguvacine is more active than THIP and γAbu as a γAbu agonist on single cells in vivo[10] and in vitro,[11] and these findings have prompted us to develop a series of esters of isoguvacine as potential prodrugs. The ideal prodrug should be sufficiently resistant to hydrolysis in the bloodstream and capable of penetrating the blood–brain barrier with subsequent hydrolysis in the brain tissue to isoguvacine.

This paper describes the syntheses of different types of esters of isoguvacine, i.e., (acyloxy)methyl esters (**5a–k**), alkyl esters (**7** and **8**), and the (ethoxycarbonyl)methyl ester (**10**) (Scheme I), designed with the purpose of obtaining a great variety in the stability toward enzymatic decomposition.

The chemical stability and the in vitro rate of hydrolysis under approximately physiological conditions are reported in Table II. A group of esters representing different rates of hydrolysis in vitro was selected and subjected to pharmacological tests in animal models known to be relevant for γAbu-ergic drugs.

Chemistry. The (acyloxy)methyl esters (**5a–k**) of isoguvacine (1) were prepared as outlined in Scheme I. The potassium salt of the *tert*-butyloxycarbonyl derivative **2** of isoguvacine was treated with the chloromethyl esters of a variety of carboxylic acids (see Table I) in boiling acetone, yielding the protected (acyloxy)methyl esters **3**

Scheme I



as oils. The crude products were deprotected by gentle acid treatment for 5 min. The hydrobromides **4**, isolated by precipitation with ether, were hygroscopic and were contaminated by small amounts of the hydrobromide of **1**, which could not be removed by recrystallization. Therefore, the hydrobromides were transformed into the fumarates **5**, which were isolated as analytically pure compounds. Two compounds, **5i** and **5j**, crystallized in some cases as the hydrogenfumarates.

The yields of **5** (Table I) were generally low, the conversion of **2** into **3** being the yield-limiting step, probably because of the low solubility of the potassium salt of **2** in acetone. Substantial amounts (30–40%) of unreacted **2** could be recovered from the reaction mixtures, but attempts to substitute the soluble triethylammonium salt of **2** for the potassium salt in the reaction sequence did not lead to the desired compounds **3**. The butyl ester **6**, however, could be prepared in high yield from the triethylammonium salt of **2** and butyl bromide. The methyl ester **8** of isoguvacine was synthesized by acid-catalyzed esterification of **1** in methanol, but this method was inapplicable with higher alcohols because of the low solubility of **1**. The (ethoxycarbonyl)methyl ester **10** was synthesized from the potassium salt of **2** and ethyl bromoacetate. The

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Table II. Half-lives (hours) for Esters of Isoguvacine at 37 °C and pH 7.35

		in 10% human serum	
no.	in 0.5 M KCl: 10 mM ester[a]	1 mM ester[a]	10 mM ester[a]
5a	10.9	0.60	1.2
5b	11.1	0.17	0.54
5c	11.7	0.06	0.25
5d	10.8	0.13	0.73
5e	12.0	0.03	0.28
5f	13.3	0.72	3.5
5g	17.1	4.6	14.9
5h	13.0	0.06	0.32
5i	15.7	2.3	7.9
5j	9.3	0.07	0.40
5k	10.0	0.03	0.14
7			600
8			430
10			300

[a] Initial concentration of ester.

halides of **7**, **8**, and **10** were isolated as analytically pure compounds.

Hydrolysis Studies. The rates of hydrolysis of the (acyloxy)methyl esters **5a–k** were examined in vitro in the absence and presence of human serum (Table II). It is assumed[13] that the esters are hydrolyzed to the hydroxymethyl ester of **1**, which subsequently decomposes spontaneously to **1** and formaldehyde. The hydrolysis was studied at pH 7.35 and 37 °C by the pH-stat technique based on the titration of the 2 acid equivalents liberated. The rate of nonenzymatic hydrolysis followed pseudo-first-order rate kinetics and was found to be very similar for all of the (acyloxy)methyl esters studied, whereas in the presence of 10% human serum great differencies were observed. Like the esters with unbranched acyl groups, the (benzoyloxy)methyl ester **5j** and the [(cyclohexylcarbonyl)oxy]methyl ester **5k** were very susceptible to enzymatic hydrolysis. The methyl ester **8**, the butyl ester **7**, and the (ethoxycarbonyl)methyl ester **10**, on the other hand, were very slowly hydrolyzed to isoguvacine.

It was found that the ester **10** is hydrolyzed in two steps. In the first step, the carboxymethyl ester of **1** is assumed to be formed, which subsequently is hydrolyzed to **1** at a much slower rate. The half-life for the first step was estimated by TLC (see Experimental Section) to be 50 h, while the half-life for the second step was approximately 300 h.

Two isomeric (acyloxy)methyl esters, **5e** and **5g**, containing unbranched and branched acyl groups, respectively, were chosen for a more detailed investigation of the relationship between the initial concentrations of the esters and their rates of hydrolysis (Figure 1). The applied technique for the determination of the rates of hydrolysis did not allow initial concentrations lower than 1.0 mM to be studied. The rate of hydrolysis of the unbranched ester **5e** in 10% human serum was found to be directly proportional to the initial concentration, while the (pivaloyloxy)methyl ester **5g** showed a linear relationship between the half-lives and the initial concentrations. In order for us to test the decomposition of the (acyloxy)methyl esters in other enzyme systems than human serum, we examined the rate of hydrolysis of 1 mM solutions of the esters **5e** and **5g** in rat brain extract. The half-lives were found to be 1.3 and 6.2 h, respectively, indicating a high degree of enzymatic hydrolysis by rat brain esterases.

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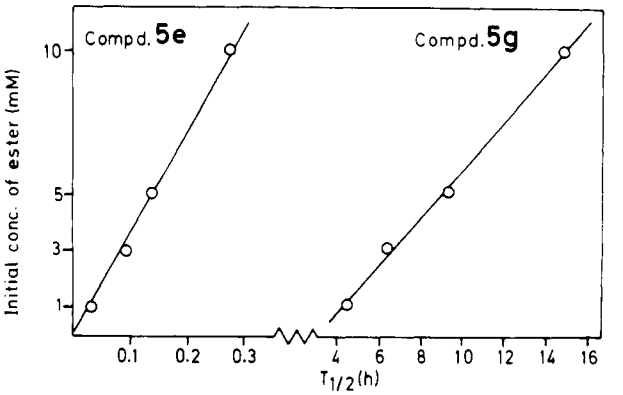

Figure 1. The half-lives as functions of the initial concentrations of ester in 10% human serum.

Table III. Anticonvulsant Activities of Esters of Isoguvacine in Mice[a]

	convulsions induced by		
no.	bicuculline	isoniazide	electroshock
1	>80	>160	>80
5a	>80	40 (0.5-2)	5 (1-2)
5b	>80	40 (0.5-1)	>80
5g	20 (6)	>160	10 (4-6)
5j	>80	>160	>80
7	40 (17)	>160	40 (17-24)

[a] Minimum effective anticonvulsant dose in milligrams per kilogram. The time (in hours) between the onset of activity and the administration (iv) of the compounds are indicated in parentheses.

Pharmacological Results and Discussion

Five esters representing different rates of hydrolysis in vitro were selected, and their effect on convulsions induced by bicuculline, isoniazide, and electroshock were determined (Table III). In order for us to attempt to estimate the time course of the absorption and decomposition of the esters, the convulsions were induced at different times (see Experimental Section) after injection of the ester.

In the bicuculline test the (pivaloyloxy)methyl ester **5g** had a weak but significant effect 6 h after administration, while the butyl ester **7** had an anticonvulsant effect initiating 17 h after administration.

In doses up to 160 mg/kg, the compounds **5g**, **5j**, and **7** had no significant effect on isoniazide-induced convulsions, while **5a** had an effect 2 h after administration and the (propionyloxy)methyl ester **5b** was effective 30 and 60 min after administration.

In comparing the results from the electroshock tests with the in vitro rate of hydrolysis (Table II), we found a good correlation between pharmacological activity and rate of enzymatic decomposition. The rapidly hydrolyzed esters **5b** and **5j** with half-lives in serum of less than 10 min have no effect, while **5a** ($T_{1/2} \approx 40$ min) had an effect after 1 h and the (pivaloyloxy)methyl ester **5g** ($T_{1/2} \approx 5$ h) had an effect 4–6 h after the administration. The activity of the very slowly hydrolyzed butyl ester **7** ($T_{1/2} \approx 400$ h) was first detectable 17 h after the injection.

The weak effects of the esters on convulsions induced by bicuculline and isoniazide could either be due to failure of the esters to enter the brain or to a low anticonvulsant activity of isoguvacine itself. It has been reported[22] that isoguvacine after intracerebroventricular administration produced only partial protection against audiogenic seizures. However, the effects on electroshock-induced convulsions indicate that the esters are capable of penetrating the blood–brain barrier. The good correlation between the

time of the onset of anticonvulsant activity and the in vitro rates of enzymatic hydrolysis suggests that the esters are hydrolyzed to isoguvacine in the brain. The maximal effects are obtained with esters having half-lives in serum in the range of 1 h to a few hours, and as a result of these observations the acetoxymethyl ester **5a**, the (pivaloyloxy)methyl ester **5g**, and the (diethylacetoxy)methyl ester **5i** will undergo further investigations.

Experimental Section

Chemistry. Melting points were determined in capillary tubes and were not corrected. NMR spectra were recorded on a Varian T-60 spectrometer by Mrs. M. Wehmeyer, Chemical Laboratory II, University of Copenhagen. Elemental analyses were performed by P. Hansen, Chemical Laboratory II, University of Copenhagen. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4%.

The syntheses of most of the chloromethyl esters have been described previously.[14-16] Chloromethyl isovalerate (bp[15] 44–50 °C), chloromethyl caproate (bp[12] 70–72 °C), and chloromethyl cyclohexanecarboxylate (bp[12] 82–86 °C) were prepared analogously.

1-(*tert*-Butyloxycarbonyl)-1,2,3,6-tetrahydropyridine-4-carboxylic Acid [2; (*tert*-Butyloxycarbonyl)isoguvacine]. To a solution of 1[17] (50 mmol) in H_2O (75 mL) was added triethylamine (TEA; 28 mL, 0.20 mol), followed by a solution of crude *tert*-butyl azidoformate[18] (8.5 mL) in dioxane (75 mL). The mixture was stirred for 24 h at room temperature. The dioxane was removed in vacuo, and the aqueous solution was cooled in an ice bath. Acidification with 1 N HCl precipitated **2**, which was collected immediately, washed with water, and dried: yield 11.0 g (97%); mp 151–152 °C. For analysis, a small amount was recrystallized from toluene with no change in the melting point. Anal. ($C_{11}H_{17}NO_4$) C, H, N.

(Acyloxy)methyl Esters of (*tert*-Butyloxycarbonyl)isoquvacine (3). General Method. To a solution of **2** (10 mmol) in acetone (200 mL) was added K_2CO_3 (15 mmol), and the mixture was stirred at room temperature for 30 min. A solution of the appropriate chloromethyl ester (11 mmol) in 10 mL of acetone was added, and the mixture was refluxed for 8 h. The reaction mixture was filtered and the filtrate evaporated in vacuo. Ether (100 mL) was added to the residue. After filtration, the ether was evaporated, leaving **3** as an oil, which was used in the next step without purification.

Hydrobromides of (Acyloxy)methyl Esters of Isoguvacine (4). General Method. An ice-cold solution of **3** (5 mmol) in EtOAc (15 mL) was treated with a 33% solution of HBr in HOAc (1.4 mL). After 5 min at room temperature, ether (300 mL) was added. The precipitate was isolated by decantation or filtration and recrystallized from EtOAc–ether or EtOH–ether.

Fumarates of (Acyloxy)methyl Esters of Isoguvacine (5a–k). General Method. To a suspension of **4** (4.0 mmol) in EtOAc (150 mL) was added a 5% aqueous solution of $NaHCO_3$ (80 mL). The layers were separated, and the aqueous phase was extracted with two 150-mL portions of EtOAc. The combined organic phases were washed with H_2O, dried, and concentrated to half volume in vacuo. A solution of fumaric acid (3 mmol) in MeOH (10 mL) was added, followed by ether (100 mL). After a few hours at 5 °C, the precipitate was collected and recrystallized from MeOH–ether (Table I).

Butyl 1-(*tert*-Butyloxycarbonyl)-1,2,3,6-tetrahydropyridine-4-carboxylate (6). To a solution of **2** (4.54 g, 20 mmol) and triethylamine (6.37 mL, 45 mmol) in 75 mL of acetone was added butyl bromide (4.31 mL, 40 mmol). The mixture was refluxed for 8 h, cooled, and filtered. The filtrate was evaporated, and EtOAc (75 mL) and H_2O (50 mL) was added to the residue. The organic layer was washed with 50 mL of a 5% solution of $NaHCO_3$ and with 50 mL of H_2O. The organic solution was dried and evaporated, leaving 5.63 g (99%) of **6** as an oil.

Butyl 1,2,3,6-Tetrahydropyridine-4-carboxylate Hydrobromide (7). A 33% solution of HBr in HOAc (5.0 mL) was added to an ice-cooled solution of **6** (5.63 g, 20 mmol) in EtOAc (40 mL). After 5 min at room temperature, ether (100 mL) was added. The precipitate was collected and recrystallized from MeOH–ether: yield 3.50 g (66%); mp 103–105 °C; NMR (D_2O) δ 0.95 (3 H, m), 1.1–1.8 (4 H, m), 2.65 (2 H, m), 3.40 (2 H, t), 3.90 (2 H, m), 4.15 (2 H, t), 6.95 (1 H, m). Anal. ($C_{10}H_{18}BrNO_2$) C, H, Br, N.

Methyl 1,2,3,6-Tetrahydropyridine-4-carboxylate Hydrochloride (8). A suspension of **1** (4.91, 30 mmol) in 250 mL of MeOH was heated, until a clear solution was obtained, and a stream of HCl gas was added during 1 h. The solution was left at room temperature over night and evaporated to dryness in vacuo. The white crystalline residue was recrystallized from acetonitrile, yielding 3.86 g (72%) of pure **8**: mp 159–161 °C; NMR (D_2O) δ 2.60 (2 H, m), 3.40 (2 H, t), 3.80 (3 H, s), 3.90 (2 H, m), 6.95 (1 H, m). Anal. ($C_7H_{12}ClNO_2$) C, H, Cl, N.

(Ethoxycarbonyl)methyl 1-(*tert*-Butyloxycarbonyl)-1,2,3,6-tetrahydropyridine-4-carboxylate (9). To a solution of **2** (1.36 g, 6.0 mmol) in acetone (85 mL) was added K_2CO_3 (1.24 g, 9.0 mmol), and the mixture was stirred for 30 min. During 10 min a solution of ethyl bromoacetate (1.1 g, 6.6 mmol) in 15 mL of acetone was added. The mixture was refluxed for 18 h and filtered. The filtrate was evaporated and the residue extracted with two 20-mL portions of ether. The ether was removed in vacuo, leaving crude **9** (1.8 g, 96%) as a yellow oil.

(Ethoxycarbonyl)methyl 1,2,3,6-Tetrahydropyridine-4-carboxylate Hydrobromide (10). To a solution of crude **9** (1.8 g, 5.7 mmol) in 30 mL of EtOAc was added a 33% solution of HBr in HOAc (1.5 mL). The mixture was left at room temperature for 15 min, and the precipitate was collected. Recrystallization from EtOH–EtOAc yielded **10** (1.04 g, 62%): mp 131–132.5 °C; NMR (D_2O) δ 1.30 (3 H, t), 2.65 (2 H, m), 3.45 (2 H, t), 3.95 (2 H, m), 4.25 (2 H, q), 4.80 (2 H, s), 7.05 (1 H, m). Anal. ($C_{10}H_{16}BrNO_4$) C, H, Br, N.

Hydrolysis Studies. Chemical Stability. The rate of hydrolysis of a 10 mM solution of the (acyloxy)methyl esters **5a–k** in 0.5 M KCl at pH 7.35 and 37 °C were followed by automatic titration with a TTT 1C titrator connected with an ABU 12 autoburette and a SBR 2C titrigraf (all from Radiometer, Copenhagen, Denmark). The ester **5** (0.5 mmol) was dissolved in 50 mL of 0.5 M KCl and 0.5 mL of a pH 7.00 phosphate buffer was added. The solution was adjusted to pH 7.35 and maintained at this value by addition of 0.5 N NaOH. The consumption of base vs. time was recorded. The measured half-lives are listed in Table II, and each value was obtained as the average of at least two experiments.

Hydrolysis in the Presence of Human Serum. The half-lives of the esters **5a–k** in 10% human serum were measured (Table II) using the same apparatus as described above. The protein content of the serum was found[19] to be in the interval 69–78 mg/mL using bovine serum albumin as standard. A stream of N_2 was passed through a mixture of 45 mL of 0.5 M KCl, 0.5 mL of phosphate buffer, and 5.0 mL of human serum for 2 h. The pH of the solution was kept at pH values between 7.2 and 7.6 by addition of 0.1 N HCl (usually a total of 1.5 mL was necessary). After this initial treatment, the pH of the reaction medium was constant for several hours. The ester (0.5 mmol or 0.05 mmol) was added and the pH was maintained at 7.35 by the addition of 0.5 N or 0.1 N NaOH, respectively. For the very labile (acyloxy)methyl esters the reaction was followed until the hydrolysis was completed, and the values of the half-lives were calculated on the basis of the consumed amount of base, which in all cases exceeded 90% of the calculated amount. The complete hydrolyses of the more stable esters, most of which were followed

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until the base consumption stopped, required between 92 and 105% of the calculated amount of NaOH.

Because of a diminutive leaking of base from the inlet, the half-lives of the very slowly hydrolyzed esters 7, 8, and 10 could not be measured by the titration technique but were estimated by TLC using butanol–HOAc–H_2O (4:1:1) as eluent. With intervals of 48 h, a sample of the hydrolysis mixture was withdrawn and applied on a silica gel plate (Merck) next to a freshly prepared solution, which was 5 mM with respect to both 1 and the ester. After development and drying, the plate was sprayed with a solution of ninhydrin in EtOH, and the intensities of the color obtained for the two spots from the hydrolysis mixture were compared with the spots from the standard solution.

Hydrolysis in Extract of Rat Brains. Six adult albino rats were decapitated and their brains were rapidly removed and homogenized in 20 mL of ice-cold 0.5 M KCl with a Warring blender for 5 min. The homogenate was dispersed with a 100-W MSE Ultrasonic disintegrator for 30 s twice with a 30-s interval. The suspension was centrifuged at 48000*g* for 20 min. The supernatant fluid was collected and made up to 30 mL by addition of 0.5 M KCl. The extract, which contained 15 mg of protein/mL,[19] was immediately divided into six portions and stored at −20 °C until needed. A mixture of 0.5 M KCl (45 mL), phoshate buffer (0.5 mL), and extract of rat brain (5.0 mL) was brought to pH 7.35, and 0.05 mmol of the ester was added. The pH was readjusted and maintained constant by the addition of 0.1 N NaOH. The hydrolysis was followed in the same manner as described above.

Pharmacology. Anticonvulsant Activity. The convulsions were induced by bicuculline, isoniazide, or by electroshock at times of 0.5, 1, 2, 3, 4, 5, 6, 17, and 24 h after injection (ip) of the ester. At each time interval, groups of at least five mice were used.

(a) Bicuculline. Bicuculline was injected in a dose of either 5 mg/kg subcutaneously or 0.63 mg/kg intravenously at each time.

(b) Isoniazide. The antagonistic effects of the esters against convulsions induced by isoniazide were determined by a previously described method.[20]

(c) Electroshock. Antagonism of maximal electroshock seizure (MES) was determined as described by Swinyard.[21] To induce the convulsions an electric high-voltage sinus wave stimulator (9.5 mA, 0.4 s) was used.

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Bioactivation of the Antitumor Drugs 9-Hydroxyellipticine and Derivatives by a Peroxidase–Hydrogen Peroxide System

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Hydroxylation in position 9 (see Table I) of various antitumor drugs derived from ellipticine results, in most cases, in the possible further oxidation of the hydroxylated drugs into free radicals and quinone products in the presence of a peroxidase–H_2O_2 system. Except for the N^6-methyl derivative, free radicals of hydroxyellipticines do not react with neighboring molecules. However, quinone products have been found to be strong electrophilic molecules. They can oxidize NADH into NAD^+ through a nonenzymatic process, and, moreover, quinone from N^2-methyl-9-hydroxyellipticine may undergo a nucleophilic attack, resulting in an irreversible binding of the drug to bovine serum albumin. Among the drugs tested, those which can be oxidized by peroxidase–H_2O_2 exhibit the most cytotoxic effect on L1210 cells in vitro.

Ellipticine [5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole; see Table I] is a plant alkaloid which exhibits a high cytotoxic activity against murine leukemia L1210 and various other tumor cells.[1] Recent experiments have identified the phenolic derivative 9-hydroxyellipticine (9-OH-E; see Table I) as a main product of the oxidative metabolism in rats.[2,3] It has been further demonstrated that the hydroxylation of ellipticine depends on the microsomal drug-metabolizing system.[4] It has been recognized for several years[5,6] that the addition of an hydroxyl group on the ellipticine ring in position 9 results in a strong increase in both cytotoxic and antitumor activities. In the field of investigation dealing with the mechanism of action of these drugs, it is interesting to determine why the hydroxylation of ellipticine will increase its antitumor activity. Since biotransformation of chemical compounds to active metabolites appears to be a common prerequisite for the development of their biological effects, one possible hypothesis is that hydroxylation of ellipticine enables it to be biotransformed into an even more reactive compound(s). Along this line, preliminary investigations[7] indicate that 9-OH-E may act as a substrate for peroxidases, enzymes which catalyze the oxidation of various arylamines and phenols in the presence of hydrogen peroxide (H_2O_2).[8] Thus, using a peroxidase–H_2O_2 system as a model of bioxidation, we have studied the ability of various ellipticines (see Table I) to be oxidized and determined, in most cases, the nature and the reactivity of the products of oxidation.

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